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(54) Anti-human milk fat globule humanised antibodies

Anti-menschliche Milchfettglobule humanisierte Antikörper

Anticorps humanisés anti-globule de matière grasse du lait

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De cripti n

FIELD OF THE INVENTION

The present invention relates to humanised antibody molecules (HAMs) having specificity for human milk fat globule (HMFG) and to processes for their production using recombinant DNA technology.

BACKGROUND TO THE INVENTION

The term "humanised antibody molecule" (HAM) is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, the remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site may comprise: either a complete variable domain from the non-human immunoglobulin fused onto one or more human constant domains; or one or more of the complementarity determining regions (CDRs) grafted onto appropriate human framework regions in the variable domain. The abbreviation "MAb" is used to indicate a monoclonal antibody.

In the description, reference is made to publications by number. These numbers are placed in square brackets [ ]. The publications are listed in numerical order at the end of the description.

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, Fab'$(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the outer end of each arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies of defined specificity [1]. However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. The resultant MAbs are therefore essentially rodent proteins. There are few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness.

Therefore proposals have been made for making non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs related to production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody are fused to constant domains derived from a second antibody. Methods for carrying out such chimerisation procedures are described in EP-A-0 120 694 (Celltech Limited), EP-A-0 125 023 (Genentech Inc.), EP-A-0 171 496 (Res. Dev. Corp. Japan), EP-A-0173494 (Stanford University) and EP-A-0 194 276 (Celltech Limited).

EP-A-0 194 276 discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. It also describes the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin and a non-immunoglobulin-derived protein in place of the Fc portion of the human immunoglobulin.

Subsequently, a number of further patent applications have been published relating to chimeric antibodies, including tumour specific chimeric antibodies. Among these applications are WO-A-87/02671 (Int. Gen. Eng. Inc.), EP-A-0 256 654 (Centocor), EP-A-0 266 663 (Int. Gen. Eng. Inc. & Oncogen), WO-A-89/00999 (Int. Gen. Eng. Inc.) and EP-A-0 332 424 (Hybritech Inc.).

Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete variable domains. Thus, such humanised antibodies may elicit some HAMA response, particularly if administered over a prolonged period [2].

In an alternative approach, described in EP-A-0 239 400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. Such CDR-grafted humanised antibodies are less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the lower proportion of non-human amino acid sequence which they contain. There are three CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

The earliest work on CDR-grafted humanised MAbs was carried out on a MAb recognising the synthetic antigen NP or NIP. However, subsequently, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising

an antigen on human T cells respectively were humanised have been described [3, 4]. The preparation of the CDR-grafted antibody to the antigen on human T cells is also described in WO-A-89/07452 (Medical Research Council). Recently the preparation of a humanised CDR-grafted antibody that binds to the interleukin 2 receptor has been described [5]. Further examples of humanised CDR-grated antibodies having specificity for anti-viral [6, 7], anti-tumour [8] and anti-T cell [9 and EP-A-0 403 156] antigens have been described more recently.

Our copending International Patent Specification No. WO-A-91/09967 relates to the CDR grafting of antibodies in general.

It has been widely suggested that immunoglobulins, and in particular MAbs, could potentially be very useful in the diagnosis and treatment of cancer [10, 11]. There has therefore been much activity in trying to produce immunoglobulins or MAbs directed against tumour-specific antigens. So far, over one hundred MAbs directed against a variety of human carcinomas have been used in various aspects of tumour diagnosis or treatment [12].

There have been a number of papers published concerning the production of chimeric monoclonal antibodies recognising cell surface antigens. For instance, genetically engineered murine/human chimeric antibodies which retain specificity for tumour-associated antigens have been described [13 and WO-A-89/01783]. Also, a recombinant murine/human chimeric monoclonal antibody specific for common acute lymphocytic leukaemia antigen has been described [14].

SUMMARY OF THE INVENTION

We have now prepared humanised antibodies to human milk fat globule (HMFG) derived from the anti-HMFG mouse MAb CTMO1 [15].

According to the present invention, there is provided a humanised antibody molecule (HAM) having specificity for human milk fat globule (HMFG) and having an antigen binding site wherein at least one of the complementarity determining regions (CDRs) of the variable domain is derived from the mouse monoclonal antibody CTMO1 (CTMO1 MAb) and the remaining immunoglobulin-derived parts of the HAM are derived from a human immunoglobulin or an analogue thereof.

The HAM may comprise a chimeric humanised antibody or a CDR-grafted humanised antibody. When the HAM comprises a CDR-grafted humanised antibody, each heavy or light chain variable domain may comprise only one or two CTMO1-derived CDRs. Preferably, however, all three heavy and light chain CDRs are derived from CTMO1.

The CTMO1 MAb is a mouse MAb of the type IgG1-kappa raised against the membrane-associated antigen of HMFG and has been extensively studied [15]. The CTMO1 MAb has been shown to recognise breast, ovarian and non-small cell lung cancers. It has been shown to internalise rapidly into target cells. Conjugates of CTMO1 and calichaemicin display highly specific cytotoxicity against appropriate cell lines, (see USP 5053394).

High levels of the antigen recognised by the CTMO1 MAb have been detected circulating in the blood of patients suffering from breast cancer. This may have a deleterious effect on pharmacokinetics and tumour localisation *in vivo*. However, circulating antigen levels in the blood of patients suffering from ovarian cancer are lower than those in breast cancer patients. It is therefore believed that the HAM of the present invention will be of particular use in the treatment of ovarian cancer.

It is believed that the CTMO1 MAb recognises the polymorphic epithelial mucin (PEM) of HMFG. Thus, preferably, the present invention provides a HAM which recognises the PEM of HMFG.

Surprisingly, it has been found that humanising the CTM01 MAb does not substantially adversely affect its binding activity or internalisation, and can create, particularly by CDR grafting, a HAM which has better binding and internalisation characteristics than the murine antibody (see Table 1 hereinafter). This produces a HAM which is of use in both therapy and diagnosis of certain human carcinomas, for example carcinomas of ovary, breast, uterus and lung.

Preferably, the HAM of the present invention is produced by recombinant DNA technology.

The HAM of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as an Fab, Fab', $(Fab')_2$ or Fv fragment; a single chain antibody fragment, e.g. a single chain Fv; a light chain or heavy chain monomer or dimer; or a fragment or analogue of any of these or any other molecule with the same specificitiy as the CTMO1 MAb.

The remaining non-CTMO1 immunoglobulin-derived parts of the HAM may be derived from a suitable human immunoglobulin. For instance, when the HAM is a CDR-grafted HAM, appropriate variable region framework sequences may be used having regard to the class or type of the CTMO1 donor antibody from which the antigen binding regions are derived. Preferably, the type of human framework used is of the same or similar class or type as the donor antibody (CTMO1 is IgG1-kappa). Advantageously, the framework is chosen to maximise or optimise homology with the donor antibody sequence, particularly at positions spatially close to or adjacent the CDRs. Examples of human frameworks which may be used to construct CDR-grafted HAMs are LAY, POM, TUR, TEI, KOL, NEWM, REI and EU [16]. KOL and NEWM are suitable for heavy chain construction. REI is suitable for light chain construction. EU is particularly suitable for both heavy chain and light chain construction. Preferably, the EU framework is used as the human frame-

work for both heavy and light chain variable domains in view of its high level of homology with the CTMO1 MAb.

The light or heavy chain variable regions of the HAM may be fused to human light or heavy chain constant domains as appropriate, (the term "heavy chain constant domains" as used herein are to be understood to include hinge regions unless specified otherwise). The human constant domains of the HAM, where present, may be selected having regard to the proposed function of the antibody, in particular the effector functions which may be required. For example, the heavy chain constant domains fused to the heavy chain variable region may be human IgA, IgG or IgM domains. Preferably human IgG domains are used. IgG1 and IgG3 isotype domains may be used when the HAM is intended for therapeutic purposes and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotype domains may be used when the HAM is intended for purposes for which antibody effector functions are not required, e.g. for imaging, diagnostic or cytotoxic targeting purposes. Light chain human constant domains which may be fused to the light chain variable region include human Lambda or, especially, human Kappa chains.

Analogues of human constant domains may alternatively be advantageously used. These include those constant domains containing one or more additional amino acids than the corresponding human domain, or those constant domains wherein one or more existing amino acids of the corresponding human domain has been deleted or altered. Such domains may be obtained, for example, by oligonucleotide directed mutagenesis. In the present invention, a particularly useful analogue of a heavy chain constant domain is an IgG4 constant domain in which a serine residue at position 241 of the corresponding naturally occuring human domain has been altered to a proline residue.

The remainder of the HAM need not comprise only protein sequences from human immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequences of a polypeptide effector or reporter molecule.

According to a second aspect of the present invention, there is provided a process for producing the HAM of the first aspect of the invention, which process comprises:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy or light chain comprising a variable domain wherein at least one of the CDRs of the variable domain is derived from the CTMO1 MAb and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light or heavy chain comprising a variable domain wherein at least one of the CDRs of the variable domain is derived from the CTMO1 MAb and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

(c) transfecting a host cell with both operons; and

(d) culturing the transfected cell line to produce the HAM.

The cell line may be transfected with two vectors, the first vector containing the operon encoding the light chain-derived polypeptide and the second vector containing the operon encoding the heavy chain-derived polypeptide. Preferably, the vectors are identical except in so far as the coding sequences and selectable markers are concerned so as to ensure as far as possible that each polypeptide chain is equally expressed.

Alternatively, a single vector may be used, the vector including the operons encoding both light chain- and heavy chain-derived polypeptides.

In further aspects, the invention also includes DNA sequences coding for the heavy and light chains of the HAM of the present invention, cloning and expression vectors containing these DNA sequences, host cells transformed with these DNA sequences and processes for producing the heavy or light chains and antibody molecules comprising expressing these DNA sequences in a transformed host cell.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se [17, 18].

The DNA sequences which encode the CTMO1 heavy and light chain variable domain amino acid sequences (and the corresponding deduced amino acid sequences) are given hereinafter in the sequence listing as Sequence ID No. 1 and Sequence ID No. 2 respectively.

DNA coding for human immunoglobulin sequences may be obtained in any appropriate way. For example, amino acid sequences of preferred human acceptor frameworks, such as LAY, POM, KOL, REI, EU, TUR, TEI and NEWM, are widely available to workers in the art. Corresponding DNA sequences which code for these amino acid sequences may be inferred or deduced by reverse application of the genetic code. Similarly, the amino acid sequences of human constant region domains are well known and DNA sequences which code for them may be readily deduced.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for CDR-grafted

products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis tech techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example, oligonucleotide directed synthesis [19] may be used. Also, oligonucleotide directed mutagenesis of a pre-existing variable domain region [3, 4] may be used. Enzymatic filling-in of gapped oligonucleotides using T4 DNA polymerase [5] may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the chimeric or CDR-grafted heavy and light chains. Bacterial, e.g. *E. coli*, and other microbial systems may be used, in particular for expression of antibody fragments, e.g. Fv, Fab and Fab' fragments and single chain antibody fragments, e.g. single chain Fvs. Eucaryotic, e.g. mammalian host cell, expression systems may be used for production of larger chimeric or CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines, for example NSO cells.

In a further aspect of the invention we provide a conjugate molecule comprising a HAM conjugated to an effector or reporter molecule. Thus for example the HAM of the present invention may have attached to it an effector molecule such as a cytotoxic or cytostatic agent, or a reporter group, for example an atom or molecule such as a radionuclide, or complexed radionuclide capable of being detected while inside the human body. For instance, the HAM may have an organic group, such as a macrocycle, capable of binding a metal atom, or a toxin, such as ricin, or an anti-tumour agent as hereinafter defined, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce a HAM in which the Fc fragment, CH2 or CH3 domain of a complete molecule has been replaced by or has attached thereto by peptide linkage a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

A particularly useful conjugate molecule according to this aspect of the invention is a HAM conjugated to a methyltrithio anti-tumour agent. Particular methyltrithio anti-tumour agents include the disulphide analogues of the α_1, α_2, α_3, α_4, β_1, β_2, γ_1, δ_1 and pseudoaglycone components of the LL-E33288 complex and derivatives thereof, as well as the disulphide analogues of BBM-1675, FR-900405, FR-900406, PD 114759, PD 115028, CL-1577A, CL-1577B, CL-1577D, CL-1577E and CL 1724 antitumour antibiotics and derivatives thereof.

The family of antibacterial and antitumor agents, known collectively as the LL-E33288 complex are described and claimed in U.S. Pat. No. 4,970,198 (1990) and are used to prepare the disulphide antitumour agents which are some of the starting materials for the conjugate molecules of the invention.

U.S. Patent 4,970,198 describes the LL-E33288 complex, the components thereof, namely, LL-E33288α_1^{Br}, LL-E33288α_1^{I}, LL-E33288α_2^{Br}, LL-E33288α_2^{I}, LL-E33288α_3^{Br}, LL-E33288α_3^{I}, LL-E33288α_4^{Br}, LL-E33288β_1^{Br}, LL-E33288β_1^{I}, LL-E33288β_2^{Br}, LL-E33288β_2^{I}, LL-E33288δ_1^{Br}, LL-E33288δ_1^{I}, LL-E33288δ_1^{I}, and methods for their production by aerobic fermentation utilizing a new strain of *Micromonospora echinospora ssp calichensis* or natural or derived mutants thereof. U.S. Pat. No. 4,970,198 also discloses proposed structures for some of the above-named components.

Additional members of the LL-E33288 complex (the calicheamicins) are described and claimed in U.S. Pat. No. 4,939,244 (1990) and are likewise useful for preparing the conjugate molecules of the invention. This patent also describes the LL-E33288 bromo- and iodo-pseudoaglycones of the series, which have been prepared by chemical means. The patent also describes dihydro derivatives accessable from all the above-named antitumor antibiotics through sodium borohydride reduction of the ketone at C_{11} to a hydroxyl group.

Still other members of the LL-E33288 family of antitumour antibiotics are described and claimed in U.S. Patent 5,079,233 (1992), and also are useful for preparing additional conjugate molecules of the invention. This patent describes N-acyl derivatives of several members of the LL-E33288 complex which have been prepared by chemical means.

Other antibiotics are useful to prepare conjugate molecules of the invention, namely:

1) Esperamicin BBM-1675, [M. Konishi, et. al., J. Antibiotics, 38, 1605 (1985); M. Konishi, et. al., U.K. Patent Specification 2,141,425A, and U.S. Pat. No. 4,675,187].

2) Antitumour antibiotics, FR-900405 and FR-900406. [M. Iwami, et. al., J. Antibiotics, 38, 835 (1985), S. Kiyoto, et. al., J. Antibiotics, 38, 340 (1985)].

3) PD 114759 and PD 115028, [R.H. Bunge, et. al., J. Antibiotics, 37, 1566 (1984) U.S. Pat. No. 4,554,162 D.W. Fry et. al., Investigational New Drugs, 4, 3 (1986)].

4) Antibiotic complex CL-1577A, CL-1566B produced by *Streptomyces* asp. ATCC 39363. U.S. Pat. No. 4,539,203 (1985).

5) CL-1577D and CL-1577E Antibiotic antitumor compounds, U.S. Pat. No. 4,539,203.

6) CL-1724 Antibiotic compounds, U.S. Pat. No. 4,554,162.

7) Antitumour antibiotics BBM-1675-A3 and BBM-1675-A4, obtained by fermentation of actinomadura verrucosospora strains H964-92 (ATCC 39334) or AB27Y (ATCC 39638). U.S. Pat. No. 4,675, 187.

8) N-acetyl-esperamicin A_1, A_2 and $A_{1\beta}$ derivatives with antimicrobial and antitumor activities. European Patent Specification 289,030.

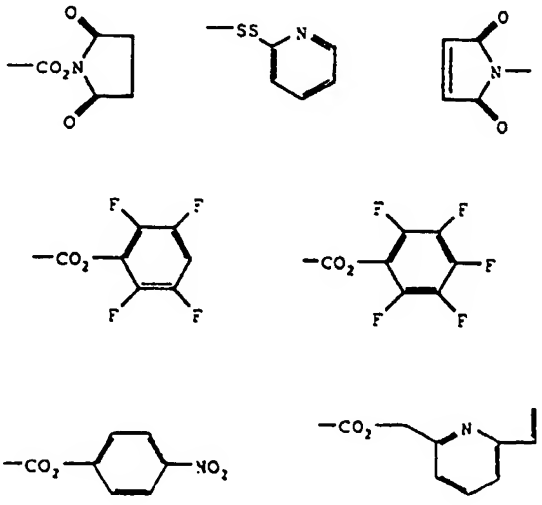
All of the information regarding the LL-E33288 family of antitumor antibiotics, BBM-1675, FR-900405, FR-900406, PD 114759, PD 115028, CL-1577A, CL-1577B, CL-1577D, CL-1577E and CL-1724 contained in the above citations is incorporated herein by reference.

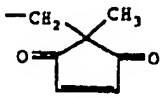
The α_1, α_2, α_3, α_4, β_1, β_2, γ_1, δ_1, and pseudoaglycone components of the LL-E33288 complex their dihydro and N-acyl counterparts, as well as the BBM-1675, FR-900405, FR-900406, PD 114759, PD 115028, CL-1577A, CL-1577B, CL-1577D, CL-1577E and CL-1724 antibiotics and their N-acyl counterparts, each contain a methyltrithio group in their structure. The methyltrithio moiety of the above-named antibiotics is subject to displacement by a variety of thiol-containing organic molecules resulting in the formation of a new class of anticancer and antibacterial agents. Displacement of the methyltrithio unit of the antitumour antibiotics as depicted in Scheme I, below, can be used to introduce a spacer (Sp), the judicious choice of which enables the introduction of a HAM of the invention (hereinafter Hu:CT-M-01) into the compounds of the above-named patents and applications to form a conjugate molecule according to the invention.

Scheme 1

$Ch_3SSS\text{-}W$ + Q-Sp-SH → Q-Sp-SS-W

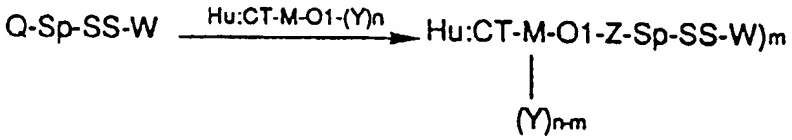
With reference to Scheme I CH_3-SSS-W is the antitumour antibiotic, Sp is a straight or branched-chain divalent or trivalent (C_1-C_{18}) radical, divalent or trivalent aryl or heteroaryl radical, divalent or trivalent (C_3-C_{18}) cycloalkyl or heterocycloalkyl radical, divalent or trivalent aryl- or heteroaryl-alkyl (C_1-C_{18}) radical, divalent or trivalent cycloalkyl- or heterocycloalkyl-alkyl (C_1-C_{18}) radical, or divalent or trivalent (C_2-C_{18}) unsaturated alkyl radical, wherein if Sp is a trivalent radical, it can be additionally substituted by amino, alkylamino, arylamino, heteroarylamino, carboxyl, lower alkoxy, hydroxy, thiol or lower alkylthio groups; Q is, or can be subsequently converted to, halogen, amino, alkylamino, carboxyl, carboxaldehyde, hydroxy, thiol, a-haloacetyloxy, lower alkyldicarboxyl, -$CONHNH_2$, -$NHCONHNH_2$, -$NHCSNHNH_2$, -ONH_2, -CON_3,



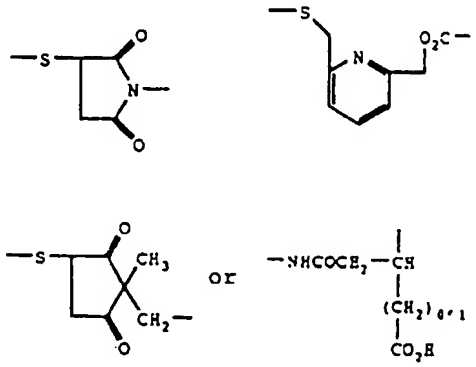


As long as the product from Scheme I contains at least one functional group which can be converted to, or is directly reactive with Hu:CT-M-01, targeted forms of the antitumor antibiotics of the above-named patents and applications can be generated, as shown in Scheme II below:

Scheme II



wherein Q, Sp, and W are as hereinbefore defined, Hu:CT-M-01 is a HAM, its fragments, or an analogue thereof; Y is a side-chain amino, carboxy, or thiol group of a protein, an aldehyde derived from carbohydrate residues, or an amidoalkylthio group; n is an integer of from 1 to 100; Z is formed from covalent reaction of the groups Q and Y directly or after subsequent reduction and Z is -CONH-, -CONHN=CH-, -CONHNHCH$_2$-, -NH-CONHN=CH-, -NHCONHNHCH$_2$-, -NHCSNHN=CH-, -NHCSNHNHCH$_2$-, -ON=CH-, -NH-, -NHCH$_2$-, -N=CH-, -CO$_2$-, -NHCH$_2$CO$_2$-, -SS-,



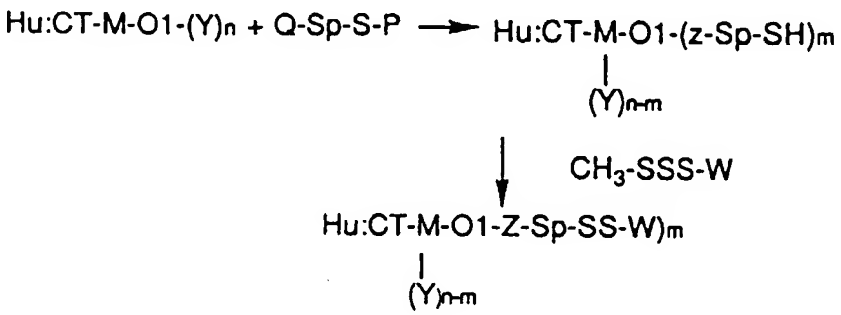
and *m* is 0.1 to 15.

As an example, and with reference to Scheme II, above, the 3-mercaptopropionic acid derivative of E-33288γ_1^I (Q=CO$_2$H, Sp=-CH$_2$CH$_2$-), when converted to its activated hydroxysuccinimide form (Q=CO$_2$Su, Sp=-CH$_2$CH$_2$-) can be used to react with some of the e-amino groups of lysine residues (e.g., Y=-NH$_2$ wherein n=50-100 from available lysine residues), of Hu:CT-M-O1 at a pH between 7.0 and 9.5 in aqueous buffered solutions at temperatures between 4°C to 40°C to produceconjugate molecules of the invention with the antibiotics attached at random sites along the protein backbone (Z=-NHCO-, Sp=-CH$_2$CH$_2$, *m*=1-10). Only a fraction of the available lysine residues are substituted in this manner, since high loading is generally not considered compatible with preserving the antibody immunoreactivity. The same randomly-substituted immunoconjugates can also be prepared from the 3-mercaptopropionic acid derivative using other carboxyl group activating agents such as a variety of carbodiimides, or the corresponding acyl azide. Alternatively, a 3-mercaptopropionyl hydrazide derivative of E-33288γ_1^I (Q=H$_2$NNHCO-, Sp=-CH$_2$CH$_2$-), when reacted with a periodate-oxidized antibody (Y=-CHO, N=1-15) as described in U.S. Pat. No. 4,671,958 at a pH between 4 and 7, in a buffered aqueous solution at a temperature of between 4°C and 40°C, reacts only at the aldehyde functionality (derived from cleavage of vic-diols of carbohydrate residues situated on the Fc portion of the antibodies) to generate Hu:CT-M-01 conjugates containing the drug substituted at specific sites along the backbone of the protein (Z=-CH=NNHCO-, Sp=-CH$_2$CH$_2$-, *m*=0.5-10). Other aldehyde-reactive groups as part of the drug construct are within our

invention to generate the products of Scheme II. Such functional groups are preferably, though not limited to, those which react with aldehydes under acidic aqueous conditions. The reactivity of protein lysines under basic conditions is sufficiently great such that their amines compete with the products of Scheme II for available aldehydes of the monoclonal antibody. Alternative aldehyde-reactive groups are for example, the semicarbazide, the thiosemicarbazide, and the O-substituted hydroxylamine functionalities.

Assembly of conjugate molecules of the invention is not restricted to the sequence outlined in Scheme II. The Hu:CT-M-01 antibody can be first modified to contain a thiol group, which is then reacted with the antitumour antibiotics useful in the invention in accordance with Scheme III below:

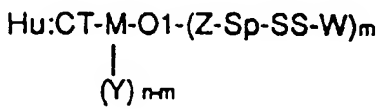
Scheme III



wherein Hu:CT-M-O1, Y, Q, Sp, W, *n*, and *m* are as hereinbefore defined, and P is hydrogen or 2-(pyridylthio), with the proviso that when Y is a thiol derived from a backbone amino acid residue of Hu:CT-M-01, Z-Sp taken together is a covalent bond.

As an example, and with references to Scheme III, above, the Hu:CT-M-01 monoclonal antibody can be reacted with 3-(2-dithiopyridyl)propionic acid hydroxysuccinimide ester to modify the protein through lysine residues (Y=NH_2, n=50-100, Q= -CO_2Su, Sp=-CH_2CH_2-, P=2-pyridylthio). Following reduction with, for example, dithiothreitol, an intermediate is generated (Z=-NHCO-, Sp=-CH_2CH_2-, P=H, m=1-15) which can be reacted with the antitumour antibiotics to generate the subject immunoconjugates. Similarly, 2-iminothiolane can be reacted with Hu:CT-M-01 to introduce thiol groups onto the surface of the protein directly, without requiring a reduction step (Z=-NHCO-, Sp=-$(CH_2)_3$-, P=H, m=1-15), and this intermediate can be reacted with the CH_3-SSS-W as before. Alternatively, sulfhydryl groups inherent within the structure of Hu:CT-M-01 in dimeric form as cystine residues can be used to participate in the reaction of Scheme III directly. Such sulfhydryls are traditionally exposed by a combination of enzymatic digestion and reduction of native monoclonal antibodies (Hu:CT-M-01 =Fab' fragment, Z-Sp=Bond, Y=SH).

A preferred embodiment of the present invention is a protein-drug conjugate of the formula:

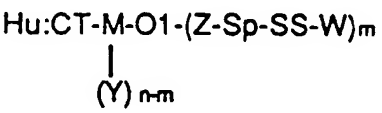


prepared from the class of antitumour antibiotics designated LL-E33288 (CH_3-SSS-W) comprising:

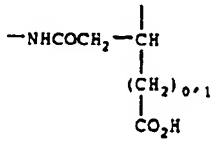
displacing the dithiomethyl moiety with a compound of formula Q-Sp-SH, wherein Sp is straight or branched-chain divalent or trivalent (C_2-C_{10}) radicals or divalent or trivalent (C_2-C_5) arylalkyl or heteroarylalkyl radicals, wherein if Sp is a trivalent radical, it can be additionally substituted by amino, heteroarylamino, hydroxy, or thiol groups; and Q is carboxyl, lower alkyldicarboxyl anhydride, -CO_2Su, -$CONHNH_2$, or

to produce an intermediate of general formula Q-Sp-SS-W, wherein Q, Sp, and W are as hereinbefore defined, and

reacting Q-Sp-SS-W with a molecule of the formula Hu:CT-M-01-$(Y)_n$ wherein Y is a side-chain amino group on the antibody, or an aldehyde generated by oxidation of the carbohydrate groups of the antibody, and *n* is an integer of from 1 to 100, to produce a compound of the formula:



wherein Y, Sp, W, and *n* are as hereinbefore defined, and Z is formed from covalent reaction of the groups Q and Y directly or after subsequent reduction, and Z is -CONH-, -CONHN=CH-, $-CONHNHCH_2-$, or



and *m* is 0.1 to 15.

The present invention also includes therapeutic and diagnostic compositions containing the HAM of the invention, particularly a conjugate molecule comprising a HAM conjugated to an effector or reporter molecule and uses of such compositions in therapy and diagnosis. Such therapeutic and diagnostic compositions typically comprise a HAM according to the invention together with a pharmaceutically acceptable excipient, diluent or carrier, e.g. for *in vivo* use.

Therapeutic and diagnostic uses typically comprise administering a pharmaceutically effective amount of a HAM according to the invention to a human subject. The exact dose to be administered will vary according to the intended use of the HAM and on the age and condition of the patient but may be typically varied from about 0.1mg to 1000mg, for example from about 1 mg to 500 mg. Me HAM may be administered as a single dose, or in a continuous manner over a period of time. Doses may be repeated as appropriate. The HAM may be formulated in accordance with conventional practice for administration by any suitable route, and may generally be in a liquid form [e.g. a solution of the antibody in a sterile physiologically acceptable buffer] for administration by for example an intravenous, intraperitoneal or intramuscular route.

In the HAM of the first aspect of the invention and the process of the second aspect of the invention, the heavy and light chain variable domains of the HAM may comprise either the entire variable domains of the CTMO1 MAb or may comprise framework regions of a human variable domain having grafted thereon one, two or all three of the CDRs of the CTMO1 MAb. Thus, the HAM may comprise a chimeric humanised antibody or a CDR-grafted humanised antibody.

When the HAM is a CDR-grafted humanised antibody, in addition to the CDRs, specific variable region framework residues may be altered to correspond to non-human, i.e. the CTMO1 mouse, residues. Preferably, the CDR-grafted humanised antibodies of the present invention include CDR-grafted humanised antibodies as defined in our International Patent Specification WO-A-91/09967.

Preferably, the CDRs of the light chain correspond to the Kabat CTMO1 MAb CDRs at CDR1 (residues 24-34) arid CDR2 (residues 50-56) and to the structural loop residues (residues 91-96) or Kabat CTMO1 MAb CDR residues (residues 89-97) in CDR3. (The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering system [16]). In addition, the light chain may have mouse CTMO1 residues at one or more of residues 1, 2, 3, 36, 37, 45, 48, 49, 60, 63, 70, 84, 85, 87 and 108. In preferred embodiments, when the human framework used is EU, the light chain comprises Kabat CTMO1 MAb CDRs at all of CDR1, CDR2 and CDR3 and preferably additional CTM01 residues at positions 3, 36, 37, 45, 48, 63 and 108, or especially additional CTMO1 residues at positions 3, 36, 63 and 108 only.

Preferably, the CDRs of the heavy chain correspond to the Kabat CTMO1 MAb CDRs at all of CDR1 (26 to 35), CDR2 (50 to 65) and CDR3 (95 - 102). In addition, the heavy chain may have mouse CTMO1 residues at one or more of residues, 2, 6, 23, 37, 48, 49, 67, 69, 73, 76, 78, 80, 88, 91 and 94. In particularly preferred embodiments, when the human framework used is EU, the heavy chain framework comprises additional CTM01 MAb residues at positions 2, 37, 71 and 73, and especially in addition at positions 48, 67 and 69.

In addition, EU has a particularly idiosyncratic J region between residues 103 to 113 and it may be useful to include the murine amino acids, a consensus human J region or a suitable combination of both at residues 103 to 108 inclusive.

When the EU framework is used, preferably heavy chain residues 94, 103, 104, 105 and 107 are murine residues, since in the case of these residues, the murine sequence is more frequently found in human VH sequences than the EU residues.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is now described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 is a schematic diagram of plasmid pRR62;
Figure 2 is a schematic diagram of plasmid pAL41;
Figure 3 is a schematic diagram of plasmid pMRR017;
Figure 4 is a schematic diagram of plasmid pHMC34;
Figure 5 is a schematic diagram of plasmid pMRR011;
Figure 6 is a schematic diagram of plasmid pHMC32;
Figure 7 is a schematic diagram of plasmid pMRR022;
Figure 8 is a schematic diagram of plasmid pMRR014;
Figure 9 is a schematic diagram of plasmid pHMC33;
Figure 10 is a schematic diagram of plasmid pMRR001;
Figure 11 is a schematic diagram of plasmid pHMC35;
Figure 12 is a schematic diagram of plasmid pHMC38;
Figure 13 is a schematic diagram of plasmid pHMC40:
Figure 14 is a schematic diagram of plasmid pHMC41;
Figure 15 is a schematic diagram of plasmid pHMC42;
Figure 16 shows the alignment of oligonucleotides H1 to H8 in the formation of the gH1 coding sequence;
Figure 17 is a schematic diagram of plasmid pAL51;
Figure 18 is a schematic diagram of plasmid pAL52;
Figure 19 is a schematic diagram of plasmid pMRR010;
Figure 20 is a schematic diagram of plasmid pAL47;
Figure 21 is a schematic diagram of plasmid pAL48;
Figure 22 is a graph of a direct binding ELISA on transiently expressed chimeric antibodies;
Figure 23 is a graph of a direct binding ELISA on transiently expressed CDR-grafted antibodies; and
Figure 24 is a graph of a competition EIA on transiently expressed chimeric and CDR-grafted antibodies.
Figure 25 is a graph comparing the effects on tumour size obtained by treating nude mice implanted with a human ovarian xenograft tumour with a humanised CDR grafted CTMO1 and a murine CTMO1 antibody each conjugated to the hydroxysuccinimide derivative of 4-mercapto-4-methyl-pentanoic acid disulphide of N-acetyl calicheamicin γ_1^I.

DESCRIPTION OF SPECIFIC EMBODIMENTS

The following description of certain embodiments of the invention is provided by way of example only and is not to be regarded as placing any limitation on the scope of the protection claimed.

MOLECULAR CLONING AND CONSTRUCTION OF THE CTMO1 CHIMERIC HEAVY CHAIN

The heavy chain variable domain of CTMO1 was cloned using the polymerase chain reaction. This enabled the construction of the chimeric version in a single step as described below.

Polyadenylated RNA was isolated from the CTMO1 hybridoma cell line using the guanidinium isothiocyanate/ lithium chloride method [17]. Double stranded cDNA was synthesised and used as a template for PCR amplification of the VH gene. A set of twenty four 5' forward primers were synthesised to complement a sequence within the murine leader sequence of VH domains [16] and to introduce a BstEII restriction site. A set of twelve 3' reverse primers was synthesised to complement the framework 4 region of VH [20] and included an ApaI restriction site.

The sequence of the basic 5' primer is given in the Sequence Listing as ID No. 3. The set of twenty four primers was based on this primer as follows. In one group of twelve primers, residue 27 remained as C. In three subgroups of four primers, residue 25 either remained as G or is altered to C or T. In each subgroup, the four primers differed at residue 28, which was A, C, G or T. In the subgroups where residue 25 is C or T, the sixth amino acid is His.

In the second group of twelve primers, residue 27 is changed to G. In three subgroups of four primers, residue 25 either remains as G or is altered to C or T. In each subgroup, the four primers differed at residue 28, which was A, C,

ʒ, amino acid 6 is Gln and where residue 25 is T, amino acid residue G is His. Where esidue 7 is Cys. Where residue 28 is G, amino acid residue 7 is Trp.
PCR primer is given in the Sequence Listing as ID No. 4. The set of twelve primers ɔws. Residue 5 could remain as G or could be altered to A or T. Residue 11 either ɛesidue 12 either remains as A or is altered to C.
VH was carried out using the following conditions:
g cDNA; 0.5 U Taq polymerase; 94°C 1 min; 50 °C 2 min; 72°C 3 min; for 40 cycles.
ıent was restricted with BstEII and ApaI and ligated to an adaptor to reconstruct the ;III restriction enzyme site. The sequence of the adaptor used is given in the Sequence ɹ codes in part for the leader amino acid sequence of the VH domain of the murine ' B72.3 (WO-A-89/01783).
ıen cloned into the HindIII/ApaI sites of the vector pE1004 to give plasmid pRR62 62 consists of an SV40 origin of replication followed by the hCMV-MIE promoter/ ıhancer controls a nucleotide sequence encoding a chimeric heavy chain comprising domain fused to human g4 constant domains. Downstream of the coding sequence

ıon of several independent clones of pRR62 were sequenced. The DNA sequence CTMO1 VH are given in Sequence ID No. 1.

ONSTRUCTION OF THE CTMO1 CHIMERIC LIGHT CHAIN

ılated from the CTMO1 hybridoma cell line using the guanidinium isothiocyanate/ ıble stranded cDNA was synthesised [21] and a cDNA library was constructed in ıkers. A screening probe was synthesised, complementary to mouse immunoglobulin :R amplification. The light chain probe was a 318 bp PCR fragment encoding the region [23].
' ($g^{32}P$) ATP by random hexanucleotide priming and was used to screen the cDNA

ɔ complete leader, variable and constant domains of light chain was isolated and

:ncodes the variable domain of the light chain was recovered by PCR amplification. stbI and SpII restriction sites at the 5' and 3' ends of the VL region respectively to fragment.
of plasmid pRB63 was restricted with BstbI/SpII and ligated between the BstbI/SpII ɔduce plasmid pAL41, which is shown in Figure 2. Plasmid pAL41 consists of a ɡ downstream of it the hCMV-MIE promoter/enhancer region. The promoter/enhancer ıence encoding a chimeric light chain comprising the CTMO1 light chain variable stant domain. Downstream of the coding sequence is a poly A site.
. was carried out according to the chain termination procedure [24]. The VH coding VL coding sequence insert in pAL41 were fully sequenced. The DNA and predicted ıocessed variable domains of the CTM01 heavy and light chains are shown in the : end of the description as Sequence ID No. 1 and No. 2 respectively.
equence coding for the VH domain and the predicted amino acid sequence. The ıin runs from residue 1 to residue 19 as shown in Sequence No. 1. Sequence No. 2 : VL domain together with the predicted amino acid sequence. The leader sequence ıe 1 to residue 20 as shown in Sequence No. 2. Examination of the derived amino ⁻able homology with other characterised immunoglobulin genes. The CTMO1 MAb ıa antibody.

NTIBODY PRODUCTS

ıR

41 carrying the hCMV promoter and chimeric light chain was cloned into plasmid : 3. Plasmid pMRR017 has a GS mini gene (WO-A-87/04462), hCMV-MIE promoter/ ıence and a poly A site. This produced plasmid pHMC34, which is shown in Figure ric light chain gene is under the control of the hCMV-MIE promoter/enhancer se-

CHIMERIC HEAVY CHAIN VECTORS

IgG1 CONSTRUCT

A HindIII-ApaI fragment containing the sequence encoding the VH domain was excised from plasmid pRR62 (Figure 1). This fragment was inserted between the HindIII and ApaI sites of plasmid pMRR011. Plasmid pMRR011 is shown in Figure 5 and comprises an hCMV-MIE promoter/enhancer region, an SV40 polyadenylation sequence, a gpt gene and a sequence encoding a human IgG1 heavy chain lacking a variable domain. The plasmid thus produced, pHMC32, is shown in Figure 6 and has a chimeric heavy chain coding sequence under the control of the hCMV-MIE promoter/enhancer. The chimeric heavy chain has the VH domain from the CTM01 MAb fused to human IgG1 constant domains.

IgG2 CONSTRUCT

The HindIII-ApaI fragment of pRR62 (Figure 1) was inserted between the HindIII and ApaI sites of a plasmid containing an hCMV-MIE promoter, a polylinker site and a nucleotide coding sequence which encodes the three constant domains of a human IgG2 antibody. This yielded plasmid pMRR022 which encodes a chimeric heavy chain having the CTM01 variable domain linked to the human IgG2 constant domains.

IgG4 CONSTRUCT

The HindIII-ApaI fragment of pRR62 (Figure 1) was inserted between the HindIII and ApaI sites of plasmid pMRR014 to produce plasmid pHMC33. Plasmids pMRR014 and pHMC33 are shown in Figure 8 and 9 respectively. Plasmid pMRR014 has an hCMV-MIE promoter, a polylinker site and a nucleotide coding sequence which encodes the three constant domains of a human IgG4 antibody. Plasmid pHMC33 is identical to plasmid pHMC32 except that the coding sequence encodes a chimeric heavy chain having the CTM01 variable domain and human IgG4 constant domains in place of the human IgG1 constant domains.

ALTERED IgG4 CONSTRUCT

The HindIII-ApaI fragment was reisolated from plasmid pHMC33. Plasmid pMRR001 shown in Figure 10 was digested with HindIII and ApaI. The large fragment was isolated and ligated to the HindIII-ApaI fragment of pHMC33 to produce plasmid pHMC35, shown in Figure 11. Plasmid pHMC35 is almost identical to plasmid pHMC32 except that the coding sequence encodes a chimeric heavy chain having the CTM01 variable domain and altered human IgG4 (hereinafter referred to as IgG4P) constant domains in place of the human IgG1 constant domains.

The alteration in the constant domains comprises a change of a serine residue in the hinge region at position 241 to a proline residue. This change advantageously abolished the formation of an 80 KD half antibody which otherwise occasionally is formed with IgG4 constant domains.

CHIMERIC HEAVY AND LIGHT CHAIN VECTORS

Vectors were constructed having operons coding for both heavy and light chains within the same vector.

A NotI-SalI fragment carrying the hCMV-MIE promoter/enhancer, the chimeric light chain encoding sequence and the SV40 poly A site together with the GS mini gene was excised from plasmid pHMC34 (Figure 4). A NotI-HindIII fragment carrying the hCMV-MIE promoter/enhancer was excised from plasmid pHMC35 (Figure 11). A HindIII-SalI fragment carrying the altered IgG4 heavy chain coding sequence and SV40 poly A site was excised from plasmid pHMC35 (Figure 11). These three fragments were ligated together to produce plasmid pHMC38, which is shown in Figure 12, and codes for expression of chimeric light chain together with the altered IgG4 chimeric heavy chain.

Plasmids pHMC32, pMRR022 and pHMC33 were digested with HindIII and EcoRI and the fragments containing the chimeric heavy chain encoding sequences were isolated. The isolated fragments were each ligated with the large HindIII-SalI fragment of pHMC38 (Figure 12) and an EcoRI-SalI fragment comprising the SV40 poly A region. The ligations produced plasmids pHMC40, pHMC41 and pHMC42 (shown in Figures 13 to 15 respectively). pHMC40 encodes a heavy chain having IgG1 constant domains. pHMC41 encodes IgG2 constant domains and pHMC42 encodes IgG4 constant domains.

PREPARATION OF CDR-GRAFTED ANTIBODY PRODUCTS

It was decided to use the EU human antibody framework [16] for carrying out the CDR-grafting. The strategy

followed for CDR-grafting was as set out in our International Patent Specification No. WO-A-91/09967.

Two CDR-grafted heavy chains were designed. In the first, gH1, all three CDRs [as defined by Kabat, ref. 16] were changed to murine residues. In addition, residues 2, 37, 71, 73, 94, 103, 104, 105 and 107, which are outside the Kabat CDRs, were also changed to murine residues. In the second, gH2, in addition to those murine residues in gH1, residues 48, 67 and 69 were changed to murine residues with a view to improving packing of the VH domain.

Two CDR-grafted light chains were also designed. In the first, gL1, all three CDRs [as defined by Kabat, ref. 16] were changed to murine residues. In addition residues 3, 36, 63 and 108, which are outside the Kabat CDRs, were changed to murine resides. In the second, gL2, in addition to those murine residues in gL1, residues 37, 45 and 48 were changed to murine residues with a view to improving packing.

A nucleotide sequence coding for the gH1 variable domain was produced by oligonucleotide assembly using oligonucleotides H1 to H8. The sequences for these oligonucleotides are given in the Sequence Listing at the end of the description under Sequence ID Nos. 6 to 13. The way in which these oligonucleotides are assembled to produce the gH1 coding sequence is shown in Figure 16. The amino acid sequence coded for by this gH1 sequence is shown in the sequence listing under Sequence ID No. 14.

A nucleotide sequence coding for the gH2 variable domain was also produced by oligonucleotide assembly using oligonucleotides H1, H2, H3A, H4, H5, H6A, H7 and H8. Oligonucleotide H3A differs from oligonucleotide H3 (Sequence ID No. 8) in that residues 55 to 57 have been changed from GTG to GCA and residues 61 to 63 have been changed from ATT to CTG. Oligonucleotide H6A differs from oligonucleotide H6 (Sequence ID No. 11) in that residues 70 to 72 have been changed from TAC to TAA. Thus, the gH2 variable domain encodes the same sequence as is shown under Sequence ID No. 14, except that at residue 67, MET has been changed to ILE; at residue 87, VAL has been changed to ALA; and at residue 89, ILE has been changed to LEU.

A nucleotide sequence coding for the gL1 variable domain was produced by oligonucleotide assembly using oligonucleotides L1 to L8. The sequences for these oligonucleotides are given in the Sequence Listing at the end of the description under Sequence ID Nos. 15 to 22. The way in which these nucleotides are assembled is similar to that shown in Figure 16 for the gH1 coding sequence (except that L is substituted for H). The amino acid sequence coded for by the assembled gL1 variable domain coding sequence is shown in the Sequence Listing under Sequence ID No. 20.

A nucleotide sequence coding for the gL2 variable domain was produced by oligonucleotide assembly using oligonucleotides L1, L2A, L3A and L4 to L8. Oligonucleotide L2A differs from oligonucleotide L2 (Sequence ID No. 16) in that residues 28 to 30 have been changed from CAG to GTA. Oligonucleotide L3A differs from oligonucleotide L3 (Sequence ID No. 17) in that residues 25 - 27 have been changed from CAG to CTC, residues 49 - 52 have been changed from AAG to CAG and residues 59 - 61 have been changed from CAT to ATC. Thus, the gL2 variable domain encodes the same sequence as is shown under Sequence ID NO. 23, except that: at residue 23, Gln has been changed to Val; at residue 62, Gln has been changed to Leu; at residue 60, Lys has been changed to G1n; and at residue 73, Met has been changed to Ile.

For gene assembly 1 pmol of H2 - H7 or L2 - L7 was mixed with 10 pmol or H1 and H8 or L1 and L8 in a 100 ml reaction with 5U Taq polymerase. A PCR reaction was done using 30 cycles (95°C, 1 min. 50°C 1 min; 72°C 1 min). The resulting fragments were cut with HindIII and ApaI for VL with Bstb1 and SPII for VH.

The nucleotide sequences coding for gH1 and gH2 were cloned as HindIII-ApaI fragments into plasmid pMRR014 (Figure 8) to produce plasmids pAL51 and pAL52 (Figure 17 and 18 respectively).

The nucleotide sequences coding for gL1 and gL2 were cloned as HindIII-ApaI fragments into plasmid pMRR010 (Figure 19) to produce plasmids pAL47 and pAL48 (Figures 20 and 21 respectively).

TRANSIENT EXPRESSION OF CHIMERIC/CHIMERIC OR CDR-GRAFTED/CHIMERIC ANTIBODIES

The following plasmids:

pHMC38, pHMC40, pHMC41 and pHMC42

and the following pairs of plasmids:

pAL47, pHMC33; pAL48, pHMC33; pAL51, pAL41; pAL52, pAL41; and pAL48, pAL41;

were each transfected or cotransfected into CHO-L761h cells for transient expression.

Assembly ELISA assays on culture supernatants resulting from the single transfected cells showed that they contained assembled antibody.

The assembly ELISA assay for quantifying antibody yields used microwell plates coated with a goat $F(ab')_2$ anti-human IgGFc. Following incubation with transfected culture supernatants, bound chimeric or CDR-grafted antibody was revealed with a horseradish peroxidase (HRP)-conjugated murine anti-human IgK antibody using tetramethyl benzidine (TMB) as the substrate. Concentrations of chimeric or CDR-grafted whole antibody in the samples were interpolated from a calibration curve generated from serial dilutions of purified chimeric B72.3 γ4 antibody [25].

BINDING ACTIVITY OF TRANSIENTLY EXPRESSED CHIMERIC OR CDR-GRAFTED ANTIBODIES

Direct binding ELISA assays for determining the binding activity of the transiently expressed antibodies were carried out as follows.

An affinity column was prepared by attaching the CTM01 MAb to a suitable chromatographic medium in conventional manner. In a first method, pooled human urine samples were applied directly to the affinity column. In a second method, human milk was subjected to low speed centrifugation to separate the cream from skimmed milk. The skimmed milk was then subjected to high speed centrifugation to produce an aqueous and a lipid component. The aqueous component was applied to the affinity column.

Once the affinity column was loaded, by either of the two methods, column fractions were eluted at high and low pHs, neutralised and assayed for reactivity with the CTM01 MAb. Fractions showing reactivity were pooled and dialysed. The pooled fractions contained the polymorphic epithelial mucin (PEM) recognised by the CTM01 MAb.

Microwell plates were coated with PEM obtained as described above. The microwells were then incubated with serial dilutions of culture supernatants. Binding of chimeric or CDR-grafted antibody was revealed and quantified by use of an HRP-conjugated murine anti-human IgK antibody.

The results of direct binding ELISA assays on the supernatants from singly transfected cells are shown in Figure 22. These assays confirm that all the supernatants contained antibodies capable of binding to PEM. No significant differences in binding activity were observed.

The direct binding ELISA assays on the supernatants from doubly transfected cells confirmed that the supernatants contained antibodies capable of binding to PEM and that the chimeric/chimeric antibody bound better than any of the CDR-grafted/chimeric antibodies.

A competition binding assay was carried out using polystyrene beads coated with PEM obtained as described above. CTM01 MAb was radiolabelled with 125I and was used to compete with the antibody produced by the pHMC40 (IgG1) transfected cells. The potency of the chimeric antibody was 84-102% that of the CTM01 MAb.

TRANSIENT EXPRESSION OF CDR-GRAFTED/CDR-GRAFTED ANTIBODIES

The following pairs of plasmids:

pAL47, pAL51; pAL47, pAL52; pAL48, pAL51; and pAL48, pAL52;

were cotransfected into CHO-L761 cells.

Direct binding assays were carried out on the culture supernatants produced by the doubly transfected cell lines. The results of these assays are shown in Figure 23, together with some results for chimeric/CDR-grafted antibodies.

From all the direct binding assays referred to above, it can be determined that the order of binding activity of the various antibodies produced by transient expression is as follows:

$$cLcH3 > gL1ch = gL1gH2 > cLgH2 = gL2H2 = gL1gH1 = gL2cH > gL2gH1.$$

(wherein:

cL = chimeric light chain;
cH = chimeric heavy chain
gL1 = CDR-grafted light chain with lowest number of amino acid changes;
gL2 = CDR-grafted light chain with highest number of amino acid changes;
gH1 = CDR-grafted heavy chain with lowest number of amino acid changes;
gH2 = CDR-grafted heavy chain with highest number of amino acid changes).

The more active variants (cLcH, gL1cH, gL1gH2 and gL2gH2) together with the CTM01 MAb were tested in a competition enzyme immunoassay (EIA). Microwell plates were coated with PEM obtained as described above. The CTM01 MAb was biotinylated and was used to compete with the four variants referred to above. Bound biotinylated CTM01 MAb was revealed and quantified using a streptavidin-HRP conjugate and TMB.

The results of the competition EIA are shown in Figure 24, which shows the same ranking of binding activity as set out above, except that the gL1cH combination shows greater activity than the cLcH combination.

It can thus be seen that chimeric, chimeric/CDR-grafted and CDR-grafted antibodies which recognise the same antigen as the CTM01 MAb have successfully been produced.

IN VITRO CELL BINDING AND INTERNALISATION OF CDR-GRAFTED CTMO1 ANTIBODIES

Stable NSO cell lines expressing gL1gH2IgG2 CTMO1 (hereinafter hu1:CTMO1) and gL1gH2IgG4P CTMO1 (hereinafter hu:CTMO1) antibody variants were made by transfecting into NSO cells by electroporation double gene expression plasmids assembled by ligating the large (7.8 kbp) Not1/BamH1 fragment of pAL47 to the 2.4 kbp Not1/Apa1 fragment from pAL52 and either a 1.9Kbp BamH1/Apa1 (partial) fragment carrying the IgG2 constant domains or a 2kbp Apa1/BamH1 fragment carrying the IgG4P constant domains as appropriate.

Antibody, purified from the supernatant of each cultured cell line by protein-A sepharose chromatography was radiolabelled (^{125}I) and incubated using a conventional continuous exposure method with either MX-1 or MCF-7 breast carcinoma cells. Radiolabelled murine CTM01 was used in all tests as a comparison. All antibodies were incubated at 2µg/million cells. The total binding of antibodies to the cells and the peak net uptake of the antibodies by the cells was determined. The results are shown in Table 1 below. With both cell lines each CDR grafted antibody exhibited better binding and internatlisation than the murine form.

TABLE 1

Antibody	Cell Line	Total Binding, 0° (molecules/cell)	Peak Net Uptake (molecules/cell)
hu1:CTMO1	MCF-7	650,000	150,000
hu:CTMO1	MCF-7	450,000	90,000
Murine CTMO1	MCF7	300,000	70,000
hu1:CTMO1	MX-1	1,200,000	150,000
hu:CTMO1	MX-1	1,100,000	150,000
Murine CTMO1	MX-1	800,000	80,000

IN VIVO ANTI-TUMOUR ACTIVITY OF A CONJUGATE OF hu:CTMO1 AND AN ANTI-TUMOUR ANTIBIOTIC

hu:CTMO1 was conjugated to the hydroxysuccinimide derivative of 4-mercapto-4-methyl-pentanoic acid disulphide of N-acetyl calicheamicin γ_1^I as follows:

SYNTHESIS OF THE 4-MERCAPTO-4-METHYL-PENTANOIC ACID DISULPHIDE DERIVATIVE OF N-ACETYL CALICHEAMICIN γ_1^I

To N-acetyl calicheamicin γ_1^I [US Patent No. 5079233] at a concentration of 2 mg/mL in acetonitrile at -15°C was added 5 molar equivalents of 4-mercapto-4-methyl-pentanoic acid and 6 molar equivalents of triethylamine. After 24 hours at -15°C the reaction was checked by C_{18}-HPLC. [If the reaction is incomplete, additional amounts of 4-mercapto-4-methyl-pentanoic acid and triethylamine are added]. Upon completion of the reaction the volatile organics were evaporated under reduced pressure and the crude product was chromatographed on Bio-Sil A using a gradient of 1 to 5% methanol in chloroform. Pure fractions as assessed by tlc were pooled and evaporated to a glass. The ^{1}H-NMR of the product was similar to N-acetyl calicheamicin γ_1^I, but was missing the absorbance for -SSSMe and exhibits absorbances for the methylpentanoic acid moiety as expected. FAB-MS gave m/z = 1478 (M + H) and 1500 (M + Na).

SYNTHESIS OF THE HYDROXYSUCCINIMIDE DERIVATIVE OF 4-MERCAPTO-4-METHYL-PENTANOIC ACID DISULPHIDE OF N-ACETYL CALICHEAMICIN γ_1^I

To the 4-mercapto-4-methyl-pentanoic acid disulphide derivative of N-acetyl calicheamicin γ_1^I described above at a concentration of 5 mg/mL in acetonitrile at ambient temperature was aded 3 molar equivalents of N-hydroxysuccinimide and 5 molar equivalents of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride. After 1 hour the reaction was checked by C_{18}-HPLC. (If the reaction is incomplete, then additional 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride is added]. Upon completion of the reaction the volatile organics were evaporated under reduced pressure and the crude product was chromatographed on Bio-Sil A using a gradient of 0 to 5% methanol in chloroform. Pure fractions as assessed by tlc were pooled and stripped to a glass. The ^{1}H-NMR was similar to that of the product described above, but with absorbances present for succinimide, as expected. FAB-MS gave m/z = 1575 (M + H) and 1597 (M + Na).

SYNTHESIS OF hu:CTM01 CONJUGATE USING THE HYDROXYSUCCINIMIDE DERIVATIVE OF 4-MERCAPTO-4-METHYL-PENTANOIC ACID DISULPHIDE OF N-ACETYL CALICHEAMICIN γ_1^I

To hu:CTM01 in phosphate buffer at a pH of about 7.4 was added 2-6 molar equivalents of the hydroxysuccinimide derivative of 4-mercapto-4-methyl-pentanoic acid disulphide of n-acetyl calicheamicin γ_1^I, described above, in dimethylformamide (DMF) such that the final concentration of DMF was 10-15%. After completion of the reaction (2-24 hours) the low-molecular-weight organic material was removed by passing through a desalting column using pH 7.4 phosphate buffer. The product was further purified by chromatography on a gel exclusion column and concentrated to give a monomeric product with an average loading of 1-3 molecules of calicheamicin derivative per molecule of antibody.

IN VIVO TEST FOR ANTITUMOUR ACTIVITY

The human ovarian xenograft tumour, OvCar3, implanted subcutaneously in nude mice was used as a test system to study the efficacy of the hu:CTM01 conjugate in vivo. A murine CTMO1 conjugate containing the same calicheamicin was also tested for comparison. Tumours were implanted subcutaneously into athymic mice and test samples were inoculated intraperitoneally (IP) at several dose levels on a q4 day x 3 schedule, starting 2-3 days after tumour implantation with 6 mice per group and 10 in each control group. Tumour mass was determined by measuring the tumour diameter once weekly during 42 days post tumour implantation. Significant antitumour activity was defined as a sustained 58% inhibition of mean tumour mass compared with untreated controls in groups with grater than 65% survivors. At both the 1 and 3 µg doses of drug equivalents the hu:CTMO1 conjugate showed significant inhibition of tumour growth (Figure 25). No deaths were noted in the 42 days observation period in any test group. In all test groups, n=6, in the control group n=10, error bars =± Standard Error Mean for each data point.

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SEQUENCE LISTING

SEQUENCE ID NO: 1.

SEQUENCE TYPE: Nucleotide with deduced protein sequence.

SEQUENCE LENGTH: 416 bases.

STRANDEDNESS: Single.

TOPOLOGY: Linear.

MOLECULE TYPE: cDNA.

ORIGINAL SOURCE ORGANISM: Murine.

IMMEDIATE EXPERIMENTAL SOURCE

NAME OF CELL LINE: Hybridoma CTM01.

PROPERTIES: Coding sequence for variable domain of heavy chain of the CTM01 monoclonal antibody.

FEATURES: Leader sequence from residues 1 to 19.

```
ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC CTG TCG GTA ACC ACA GGT
-19 -18 -17 -16 -15 -14 -13 -12 -11 -10 -9  -8  -7  -6  -5  -4
Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly

GTC CAT TGC CAG ATC CAG CTG CAG CAG TCT GGA CCT GAG CTG GTG AAG
-3  -2  -1   1   2   3   4   5   6   7   8   9   10  11  12  13
Val His Cys Gln Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys

CCT GGG GCT TCA GTG AAG ATA TCC TGC AAG GCT TCT GGC TAC ACC TTC
14  15  16  17  18  19  20  21  22  23  24  25  26  27  28  29
Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe
```

```
ACT GAC TAC TAT ATA AAC TGG ATG AAG CAG AAG CCT GGA CAG GGA CAG CTT  192
 30  31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
Thr Asp Tyr Tyr Ile Asn Trp Met Lys Gln Lys Pro Gly Gln Gly Leu   64

GAG TGG ATT GGA TGG ATT GAT CCT GGA AGC GGT AAT ACT AAG TAC GGA AAT  240
 46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
Glu Trp Ile Gly Trp Ile Asp Pro Gly Ser Gly Asn Thr Lys Tyr Gly Asn   80

GAG AAG TTC AAG GGC AAG GCC ACA TTG ACT GTA GAC ACA TCC TCC AGC  288
 61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76
Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser   96

ACA GCC TAC ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC ACT GCT GTC  336
 77  78  79  80  81  82  83  84  85  86  87  88  89
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val  112

TAT TTC TGT GCA AGA GAG AAA ACG ACC TAT TAC TAT GCT ATG GAC TAC  384
 90  91  92  93  94  95  96  97  98  99 100 101 102
Tyr Phe Cys Ala Arg Glu Lys Thr Thr Tyr Tyr Ala Met Asp Tyr  128

TGG GGT CAA GGA ACC TCA GTC ACT GTC TCC GC  416
103 104 105 106 107 108 109 110 111 112 113
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ala  139
```

SEQUENCE ID NO: 2.

SEQUENCE TYPE: Nucleotide with deduced protein sequence.

SEQUENCE LENGTH: 399 bases.

STRANDEDNESS: Single.

TOPOLOGY: Linear.

MOLECULE TYPE: cDNA.

ORGINAL SOURCE ORGANISM: Murine.

IMMEDIATE EXPERIMENTAL SOURCE

NAME OF CELLINE: Hybridoma CTM01.

PROPERTIES: Coding sequence for variable domain of light chain of the CTM01 monoclonal antibody

FEATURES: Leader sequence from residues 1 to 20.

```
ATG AGG TGC CTA GCT GAG TTC CTG GGG CTG CTT GTG CTC TGG ATC CCT    48
-20 -19 -18 -17 -16 -15 -14 -13 -12 -11 -10  -9  -8  -7  -6  -5
Met Arg Cys Leu Ala Gly Phe Leu Gly Leu Leu Val Leu Trp Ile Pro    16

GGA GCC ATT GGG GAT ATT GTG ATG ACT CAG GCT GCA CCC TCT GTT CCT    96
 -4  -3  -2  -1   1   2   3   4   5   6   7   8   9  10  11  12
Gly Ala Ile Gly Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro    32

GTC ACT CCT GGA GAG TCA TTA TCC ATT TCC TGC AGG TCT AGT AAG AGT   144
 13  14  15  16  17  18  19  20  21  22  23  24  25  26  27  28
Val Thr Pro Gly Gly Ser Leu Ser Ile Ser Cys Arg Ser Ser Lys Ser    48

CTC CTT CAT AGT AAT GGC GAC ACT TTC TTG TAT TGG TTC CTG CAG AGG   192
 29  30   a   b   c   d   e  31  32  33  34  35  36  37  38  39
Leu Leu His Ser Asn Gly Asp Thr Phe Leu Tyr Trp Phe Leu Gln Arg    64
```

```
CCA GGC CAG TCT CCT CAA CTC CTG ATA TAT CGG ATG TCC AAC CTT GCC      240
 40  41  42  43  44  45  46  47  48  49  50  51  52  53  54  55
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala

TCC GGA GTC CCA GAC AGG TTC AGT GGC AGT GGG TCA GGA ACT TTC      288
 56  57  58  59  60  61  62  63  64  65  66  67  68  69  70  71
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Phe

ACA CTG AGA GTC AGT AGA GTG GAG GCT GAT GGT GTT TAT TAC      336
 72  73  74  75  76  77  78  79  80  82  84  85  86  87
Thr Leu Arg Val Ser Arg Val Glu Ala Asp Gly Val Tyr Tyr

TGT ATG CAA CAT CTA GAA TAT CCT TTC ACG TTC GGT GCT GGG ACC AAG      384
 88  89  90  91  92  93  94  95  96  97  98  99 100 101 102 103
Cys Met Gln His Leu Gly Tyr Pro Phe Thr Phe Gly Ala Gly Thr Lys

CTG GAG CTG AAA CGG      399
104 105 106 107 108
Leu Gly Leu Lys Arg      133
```

SEQUENCE ID NO. 3
SEQUENCE TYPE: Nucleotide sequence with corresponding amino acid sequence
SEQUENCE LENGTH: 28 bases
STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULAR TYPE: Synthesised DNA
PROPERTIES: 5' forward primers for PCR amplification of murine VH domains
FEATURES: Complementary to leader sequence of murine VH domains with introduced BstEII restriction site at residues 7 to 13

GGTGGCG GTA ACC ACA GGT GTC CAG TCA 28
Val Thr Thr Gly Val Gln Ser 7

SEQUENCE ID NO. 4
SEQUENCE TYPE: Nucleotide sequence
SEQUENCE LENGTH: 36 bases
STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthesised DNA
PROPERTIES: 3' reverse primer for PCR amplification of murine VH domains
FEATURES: Complementary to framework 4 region of murine VH including an ApaI restriction site at residues 25 to 30

AGTGGCAGAG AAGTCGGAGT TGCTTCCCGG GTAGAC 36

SEQUENCE ID NO. 5

SEQUENCE TYPE: Nucleotide sequences with corresponding amino acid sequence

SEQUENCE LENGTH: 50 bases (sense strand) and 51 bases (anti-sense strand)

STRANDEDNESS: Double

TOPOLOGY: Linear

MOLECULE TYPE: Syntheised DNA

PROPERTIES: Leader sequence adaptor for murine VH domain

FEATURES: Comprises a HindIII-BstEII fragment in part coding for the leader sequence of the VH domain of murine monoclonal antibody B72.3

```
AGCTTGCCGC CACC ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC CTG TCG        50
    ACGGCG GTGG TAC CTT ACC TCG ACC CAG AAA GAG AAG AAG GAC AGC CATTG  51
           Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser        12
```

SEQUENCE ID NO: 6
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 21.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide
PROPERTIES: Used for assembly of CDR-grafted heavy chain
FEATURES: HindIII site at residues 7-12

GCGCGCAAGC TTGCCGCCAC C 21

SEQUENCE ID NO: 7.
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 96.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted heavy chain.

TCTCAGATTC AGCTGGTGCA GTCTGGAGCA GAGGTGAAGA AGCCTGGATC 50
TTCTGTGAAG GTGTCTTGTA AGGCATCTGG ATACACCTTC ACCGAC 96

SEQUENCE ID NO: 8.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 96.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted heavy chain.

TGGATTGACC CTGGATCTGG AAATACAAAG TACAATGAGA AGTTCAAGGG 50
AAGAGTGACA ATTACAGTGG ACACATCCAC GAATACCGCC TACATG 96

SEQUENCE ID NO: 9.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 89.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted heavy chain.
FEATURES: ApaI site at residues 78-83

GAGAAGACCA CCTACTACTA CGCAATGGAC TACTGGGGAC AGGGAACACT 50
GGTGACAGTG TCTTCTGCCT CAACGAAGGG CCCGCGCGC 89

SEQUENCE ID NO: 10.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 96.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted heavy chain.

CTGCACCAGC TGAATCTGAG AATGGACTCC TGTAGTTACT GACAGGAAGA 50
AGAGAAAGAC CCAGCTCCAT TCCATGGTGG CGGCAAGCTT GCGCGC 96

SEQUENCE ID NO: 11.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 96.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted heavy chain.

TCCAGATCCA GGGTCAATCC ATCCCATCCA CTCGAGTCCC TGTCCAGGTG 50
CCTGTCTCAT CCAATTAATG TAGTAGTCGG TGAAGGTGTA TCCAGA 96

SEQUENCE ID NO: 12.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 93.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted heavy chain.

GTAGTAGTAG GTGGTCTTCT CTCTTGCACA GAAGTAGAAT GCTGTGTCCT 50
CAGATCTCAG AGAAGACAGC TCCATGTAGG CGGTATTCGT GGA 93

SEQUENCE ID NO: 13.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 21.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted heavy chain.
FEATURES: ApaI site at residues 7 - 12.

GCGCGCGGGC CCTTCGTTGA G 21

SEQUENCE ID NO: 14.

SEQUENCE TYPE: Amino acid.

SEQUENCE LENGTH: 139.

MOLECULE TYPE: Immunoglobulin heavy chain variable domain.

FEATURES: CDRs at residues 45 - 54, 69 - 85 and 118 - 128

-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	
Met	Glu	Trp	Ser	Trp	Val	Phe	Leu	Phe	Phe	Leu	Ser	Val	Thr	Thr	Gly	16
-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	
Val	His	Ser	Gln	Ile	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	32
14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
Pro	Gly	Ser	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	48
30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	
Thr	Asp	Tyr	Tyr	Ile	Asn	Trp	Met	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	64
46	47	48	49	50	51	52	52A	52B	52C	53	54	55	56	57	58	
Glu	Trp	Met	Gly	Trp	Ile	Asp	Pro	Gly	Ser	Gly	Asn	Thr	Lys	Tyr	Asn	80
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	
Glu	Lys	Phe	Lys	Gly	Arg	Val	Thr	Ile	Thr	Val	Asp	Thr	Ser	Thr	Asn	96
77	78	79	80	81	82	83	84	C	85	86	85	87	88	89		
Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Phe	112
90	91	92	93	94	95	96	97	98	99	100	0	D	C	101	102	
Tyr	Phe	Cys	Ala	Arg	Glu	Lys	Thr	Thr	Tyr	Tyr	Tyr	Ala	Met	Asp	Tyr	128
103	104	105	106	107	108	109	110	111	112	113						
Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser						139

SEQUENCE ID NO: 15.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 21.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted light chain.
FEATURES: BstBI site at residues 7 to 11

GGACTGTTCG AAGCCGCCAC C 21

SEQUENCE ID NO: 16.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 81.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted light chain.

TGGCTTACAG ATGCCAGATG CGATATCCAG ATGACTCAGA GTCCAAGTAC 50
TCTCAGTGCC AGTGTAGGTG ATAGGGTCAC C 81

SEQUENCE ID NO: 17.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 90.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted light chain.

GGTGACACCT TCCTCTATTG GTTCCAGCAG AAACCAGGTA AAGCCCCAAA 50
GCTCCTCATG TATAGGATGA GTAACCTCGC CAGTGGTGTA 90

SEQUENCE ID NO: 18.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 99.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted light chain

CAGCCAGATG ATTTCGCCAC TTATTATTGT ATGCAGCATC TCGAATATCC 50
ATTCACTTTC GGTCAGGGTA CTAAAGTAGA AGTAAAACGT ACGGGCCGG 99

SEQUENCE ID NO: 19.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 81.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted light chain.
FEATURES: BstBI site at residues 70 to 75.

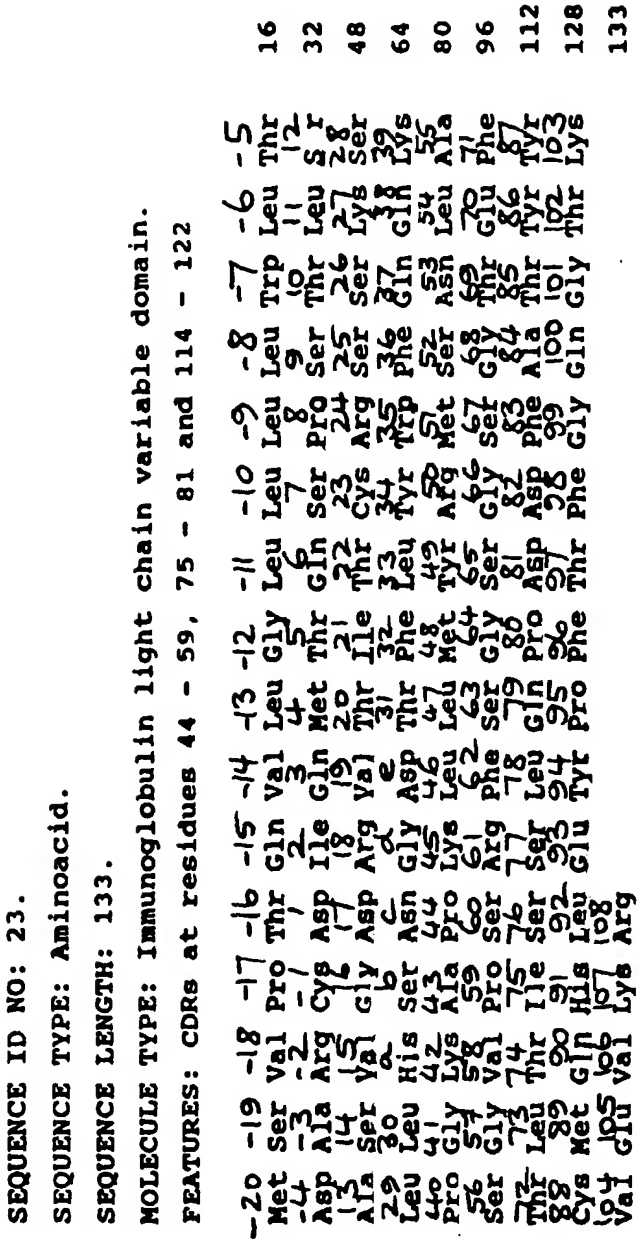
GCATCTGGCA TCTGTAAGCC ACAGCAGCAG GAGTCCGAGG ACTTGGGTGG 50
GGACAGACAT GGTGGCGGCT TCGAACAGTC C 81

SEQUENCE ID NO: 20.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 81.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted light chain.
CCAATAGAGG AAGGTGTCAC CGTTACTATG GAGGAGACTT TTACTACTCC 50
TACAAGTGAT GGTGACCCTA TCACCTACAC T 81

SEQUENCE ID NO: 21.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 102.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted light chain.

AGTGGCGAAA TCATCTGGCT GGAGACTACT GATAGTGAGA GTGAACTCAG 50
TACCACTACC ACTACCACTG AATCTAGATG GTACACCACT GGCGAGGTTA 100
CT 102

SEQUENCE ID NO: 22.
SEQUENCE TYPE: Nucleotide.
SEQUENCE LENGTH: 21.
STRANDEDNESS: Single.
TOPOLOGY: Linear.
MOLECULE TYPE: Synthetic oligonucleotide.
PROPERTIES: Used for assembly of CDR-grafted light chain.
FEATURES: SplI site at residues 7 - 12.
CCGGCCCGTA CGTTTTACTT C 21



Claims

1. An antibody molecule having specificity for human milk fat globule (HMFG) comprising a composite heavy chain and a complementary light chain wherein:

   the variable domain of said composite heavy chain comprises predominantly framework region residues from a human immunoglobulin or an analogue thereof;
   amino acid residues 2, 26 to 35, 37, 50 to 65, 71, 73, 95 to 105 and 107 (according to the Kabat numbering system) at least in said heavy chain variable domain are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in sequence ID No.1); and
   the remaining immunoglobulin derived parts of the heavy chain are derived from a human immunoglobulin or an analogue thereof.

2. An antibody molecule having specificity for human milk fat globule (HMFG) comprising a composite heavy chain and a complementary light chain wherein:

the variable domain of said composite heavy chain comprises predominantly framework region residues from a human immunoglobulin or an analogue thereof;
amino acid residues 2, 26 to 35, 37, 48, 50 to 65, 67, 69, 71, 73, 95 to 105 and 107 (according to the Kabat numbering system) at least in said heavy chain variable domain are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in sequence ID No.1); and
the remaining immunoglobulin derived parts of the heavy chain are derived from a human immunoglobulin or an analogue thereof.

3. The antibody molecule of claim 1 or claim 2, wherein additionally in said composite heavy chain at least one of residues 6, 23, 49, 76, 78, 80, 88 and 91 are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in sequence ID No.1).

4. The antibody molecule of any one of claims 1 to 3, wherein the complementary light chain is a composite light chain wherein:

the variable domain of said composite light chain comprises predominantly framework region residues from a human immunoglobulin or an analogue thereof;
amino acid residues 3, 24 to 34, 36, 50 to 56, 63, 91 to 96 and 108 (according to the Kabat numbering system) at least in said light chain variable domain are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in sequence ID No. 2); and
the remaining immunoglobulin derived parts of the light chain are derived from a human immunoglobulin or an analogue thereof.

5. The antibody molecule of any one of claims 1 to 3, wherein the complementary light chain is a composite light chain wherein:

the variable domain of said composite light chain comprises predominantly framework region residues from a human immunoglobulin or an analogue thereof;
amino acid residues 3, 24 to 34, 36, 37, 45, 48, 50 to 56, 63, 91 to 96 and 108 (according to the Kabat numbering system) at least in said light chain variable domain are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in sequence ID No. 2); and
the remaining immunoglobulin derived parts of the light chain are derived from a human immunoglobulin or an analogue thereof.

6. An antibody molecule having specificity for human milk fat globule (HMFG) comprising a composite light chain and a complementary heavy chain wherein:

the variable domain of said composite light chain comprises predominantly framework region residues from a human immunoglobulin or an analogue thereof;
amino acid residues 3, 24 to 34, 36, 50 to 56, 63, 91 to 96 and 108 (according to the Kabat numbering system) at least in said light chain variable domain are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in sequence ID No. 2); and
the remaining immunoglobulin derived parts of the light chain are derived from a human immunoglobulin or an analogue thereof.

7. An antibody molecule having specificity for human milk fat globule (HMFG) comprising a composite light chain and a complementary heavy chain wherein:

the variable domain of said composite light chain comprises predominantly framework region residues from a human immunoglobulin or an analogue thereof;
amino acid residues 3, 24 to 34, 36, 37, 45, 48, 50 to 56, 63, 91 to 96 and 108 (according to the Kabat numbering system) at least in said light chain variable domain are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in sequence ID No. 2); and
the remaining immunoglobulin derived parts of the light chain are derived from a human immunoglobulin or an analogue thereof.

8. The antibody molecule of any one of claims 4 to 7, wherein additionally in said composite light chain, residues 89, 90 and 97 are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in sequence ID No. 2).

9. The antibody molecule of any one of claims 4 to 8 wherein additionally in said composite light chain, at least one of residues 1, 2, 49, 60, 70, 84, 85 and 87 are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in sequence ID No. 2).

10. The antibody molecule of any one of claims 1 to 9, wherein the human framework residues in the composite light chain variable domain and/or composite heavy chain variable domain are derived from the human LAY, POM, TUR, TEI, KOL, NEWM, REI or EU variable domain sequences.

11. The antibody molecule of claim 10, wherein the human framework residues are derived from the human EU sequence.

12. The antibody molecule of any one of claims 1 to 11, which is a complete immunoglobulin.

13. The antibody molecule of claim 12, wherein the constant region of the heavy chain is of human IgG class.

14. The antibody molecule of claim 13, wherein the constant region of the heavy chain is of human IgG4 subclass.

15. The antibody molecule of claim 13, wherein the constant region of the heavy chain is of human IgG4 subclass with a proline residue at position 241.

16. The antibody molecule of any one of claims 1 to 11, which is a Fab, Fab' or $F(ab')_2$ fragment.

17. The antibody molecule of any one of claims 12 to 16, wherein the constant domain of the light chain is of the human kappa class.

18. The antibody molecule of any one of claims 1 to 11, which is an Fv or single chain Fv fragment.

19. The antibody molecule of any one of claims 1 to 18, which is produced by recombinant DNA technology.

20. A process for producing the antibody molecule of any one of claims 1 to 19, which process comprises:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody molecule heavy chain as defined in any one of claims 1 to 19;
(b) producing in an expression vector an operon having a DNA sequence which encodes an antibody molecule light chain as defined in any one of claims 1 to 19;
(c) transfecting a host cell with the or each vector; and
(d) culturing the transfected cell line to produce the antibody molecule.

21. The process of claim 20, for the production of an antibody fragment according to claim 16 or claim 18, in which the host cell is a bacterial host cell.

22. The process of claim 20, in which the host cell is a mammalian host cell.

23. A conjugate molecule comprising the antibody molecule of any one of claims 1 to 19 conjugated to an effector molecule or to a reporter molecule.

24. A conjugate molecule according to Claim 23 wherein the effector molecule is a methyltrithio anti-tumour agent.

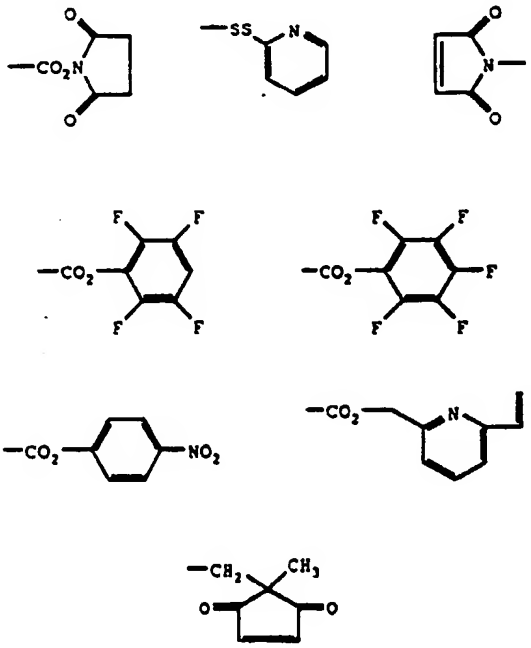
25. A conjugate molecule according to Claim 24 wherein the methyltrithio anti-tumour agent is a disulphide analogue of the α_1, α_2, α_3, α_4, β_1, β_2, γ_1, δ_1 and pseudoaglycone components of the LL-E33288 complex and derivatives thereof, BBM-1675, FR-900405, FR-900406, PD 114759, PD 115028, CL-1577A, CL-1577B, CL-1577D, CL-1577E and CL 1724 antitumour antibiotics and derivatives thereof.

26. A conjugate molecule according to Claim 25 of the formula

$$\text{Hu:CT-M-O1-(Z-Sp-SS-W)}_m \\ \Big|_{(Y)_{n-m}}$$

prepared from a compound of formula CH3-SSS-W wherein CH_3-SSS-W is an antitumour antibiotic designated as LL-E33288α_1^{Br}, α_1^{I}, α_2^{Br}, α_3^{Br}, α_3^{Br}, α_4^{Br}, β_1^{Br}, β_1I, β_2^{Br}, β_2^{I}, γ_1^{Br}, γ_1^{Br}, γ_1^{I}, δ_1^{I}, the iodo or bromo pseudoaglycone, their dihydro or N-acyl counterparts, BBM-1675, FR-900405, FR-900406, PD 114759, PD 115028, CL-1577A, CL-1577B, CL-1577D, CL-1577E, CL 1724 or their N-acetyl counterparts comprising:

reacting CH_3-SSS-W with a compound of general formula Q-Sp-SH, wherein Sp is a straight or branched-chain divalent or trivalent (C_1-C_{18}) radical, divalent or trivalent aryl or heteroaryl radical, divalent or trivalent (C_3-C_{18}) cycloalkyl or heterocycloalkyl radical, divalent or trivalent aryl- or heteroaryl-alkyl (C_1-C_{18}) radical, divalent or trivalent cycloalkyl- or heterocycloalkyl-alkyl (C_1-C_{18}) radical or divalent or trivalent (C_2-C_{18}) unsaturated alkyl radical, wherein if Sp is a trivalent radical, it can be additionally substituted by amino, alkylamino, arylamino, heteroarylamino, carboxyl, lower alkoxy, hydroxy, thiol, or lower alkylthio groups; and Q is, or can be subsequently converted to, halogen, amino, alkylamino, carboxyl, carboxaldehyde, hydroxy, thiol, a-haloacetyloxy, lower alkyldicarboxyl, -CONHNH$_2$, -NHCONHNH$_2$, -NHCSNHNH$_2$, -ONH$_2$, -CON$_3$,

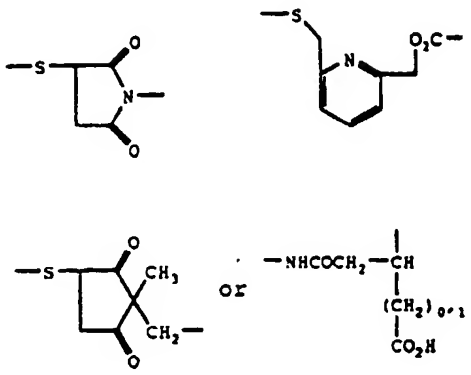


to produce an intermediate formula of the formula Q-Sp-SS-W, wherein Q, Sp, and W are as hereinbefore defined,
reacting Q-Sp-SS-W with a molecule of the formula Hu:CT-M-01-$(Y)_n$ wherein Hu:CT-M-O1 is a HAM according to Claims 1 to 17 and Y is a side-chain amino, carboxyl, or thiol group of a protein, an aldehyde derived from glycoprotein carbohydrate residues, or an amidoalkylthio group; and *n* is an integer of from 1 to 100, to produce a compound of the formula:

$$\text{Hu:CT-M-O1-(Z-Sp-SS-W)}_m \\ \Big|_{(Y)_{n-m}}$$

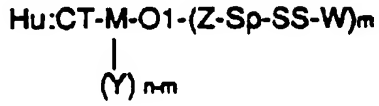
wherein Y, Sp, W, an *n* are as hereinbefore defined, and Z is formed from covalent reaction of the groups Q

and Y directly or after subsequent reduction, and Z is -CONH-, -CONHN=CH-, -CONHNHCH$_2$-, -NHCS-NHN=CH-, -NHCH$_2$-, -N=CH-, -CO$_2$-, -NHCH$_2$CO$_2$-, -SS-,



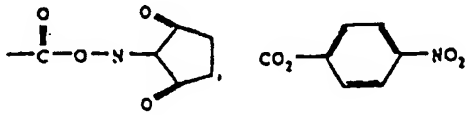
and *m* is 0.1 to 15.

27. A conjugate according to Claim 26 of the formula



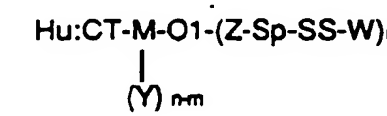
prepared from the class of antitumour antibiotics designated LL-E33288 (CH$_3$-SSS-W) comprising:

displacing the dithiomethyl moiety with a compound of formula Q-Sp-SH, wherein Sp is straight or branched-chain divalent or trivalent (C$_2$-C$_{10}$) radicals or divalent or trivalent aryl- or heteroarylalkyl (C$_2$-C$_5$) radicals, wherein if Sp is a trivalent radical, it can be additionally substituted by amino, heteroarylamino, hydroxy, or thiol groups; and Q is, or can be subsequently converted to, carboxyl, lower alkyldicarboxyl anhydride, -CONHNH$_2$, or

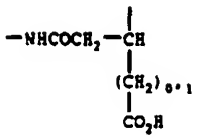


to produce an intermediate of general formula Q-Sp-SS-W, wherein Q, Sp, and W are as hereinbefore defined,

reacting Q-Sp-SS-W with a molecule of the formula Hu:CT-M-01-(Y)n wherein Y is a side-chain amino group on the antibody, or an aldehyde generated by oxidation of the carbohydrate groups of the antibody, and *n* is an integer of from 1 to 100, to produce a compound of the formula:



wherein Y, Sp, W, and *n* are as hereinbefore defined, and Z is formed from covalent reaction of the groups Q and Y directly or after subsequent reduction, and Z is -CONH-, -CONHN=CH-, -CONHNHCH$_2$-, or



and *m* is 0.1 to 15.

28. A conjugate according to Claim 27 wherein CH_3-SSS-W is the antitumour antibiotic designated LL-E33288γ_1^I.

29. A conjugate according to Claim 27 wherein CH_3-SSS-W is the antitumour antibiotic designated LL-E33288α_2^I.

30. A conjugate according to Claim 27 wherein CH_3-SSS-W is the antitumour antibiotic designated LL-E33288α_3^I.

31. A conjugate according to Claim 27 wherein CH_3-SSS-W is the antitumour antibiotic designated N-acetyl LL-E33288γ_1^I.

32. A conjugate according to Claim 27 wherein CH_3-SSS-W is the antitumour antibiotic designated iodo LL-E33288 pseudoaglycone.

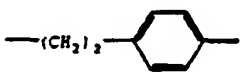
33. A conjugate according to Claim 27 wherein Q is the hydroxysuccinimide ester of a carboxyl group, Sp is -CH_2CH_2-, Y is -NH_2, Z is -CONH-, and *m* is 0.5 to 15.

34. A conjugate according to Claim 27 wherein Q is the hydroxysuccinimide ester of a carboxyl group, Sp is -$CH_2CH(CH_3)$-, Y is -NH_2, Z is -CONH-, and *m* is 0.5 to 15.

35. A conjugate according to Claim 27 wherein Q is the 4-nitrophenyl ester of a carboxyl group, Sp is -CH_2CH_2-, Y is -NH_2, Z is -CONH-, and *m* is 0.5 to 15.

36. A conjugate according to Claim 27 wherein Q is the hydroxysuccinimide ester of a carboxyl group, Sp is -$CH_2C(CH_3)_2$-, Y is -NH_2, Z is -CONH-, and *m* is 0.5 to 15.

37. A conjugate according to Claim 27 wherein Q is the hydroxysuccinimide ester of a carboxyl group, Sp is



Y is -NH_2, Z is -CONH-, and *m* is 0.5 to 15.

38. A conjugate according to Claim 27 wherein Q is -$CONHNH_2$, Sp is -CH_2CH_2-, Y is -CHO, Z is -CONHN=CH-, and *m* is 0.1 to 10.

39. A conjugate according to Claim 27 wherein Q is -CONHNH2, Sp is -CH_2CH_2-, Y is -CHO, Z is -$CONHNHCH_2$-, and *m* is 0.1 to 10.

40. A conjugate according to Claim 27 wherein Q is -$CONHNH_2$, Sp is -$CH_2CH(CH_3)$-, Y is -CHO, Z is -CONHN=CH-, and *m* is 0.1 to 10.

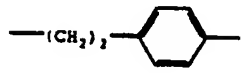
41. A conjugate according to. Claim 27 wherein Q is -$CONHNH_2$, Sp is -$CH_2CH(CH_3)$-, Y is -CHO, Z is -$CONHNHCH_2$-, and *m* is 0.1 to 10.

42. A conjugate according to Claim 27 wherein Q is -$CONHNH_2$, Sp is -$CH_2C(CH_3)_2$-, Y is -CHO, Z is -CONHN=CH-, and mis 0.1 to 10.

43. A conjugate according to Claim 27 wherein Q is -$CONHNH_2$, Sp is -$CH_2C(CH_3)_2$-, Y is -CHO, Z is -$CONHNHCH_2$-,

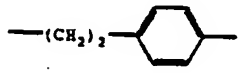
and *m* is 0.1 to 10.

44. A conjugate according to Claim 27 wherein Q is -CONHNH$_2$, Sp is



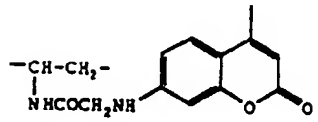
Y is -CHO, Z is -CONHN=CH-, and *m* is 0.1 to 10.

45. A conjugate according to Claim 27 wherein Q is -CONHNH$_2$, Sp is



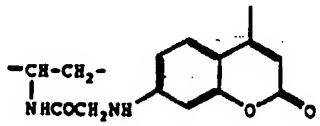
Y is -CHO, Z is -CONHNHCH$_2$-, and *m* is 0.1 to 10.

46. A conjugate according to Claim 27 wherein Q is -CONHNH$_2$, Sp is



Y is -CHO, Z is -CONHN=CH-, and *m* is 0.1 to 10.

47. A conjugate according to Claim 27 wherein Q is -CONHNH$_2$, Sp is



Y is -CHO, Z is -CONHNHCH$_2$-, and *m* is 0.1 to 10.

48. A conjugate according to Claim 38 wherein CH$_3$-SSS-W is LL-E33288γ_1^I, Q is -CONHNH$_2$, Sp is -CH$_2$CH$_2$-, Y is -CHO, Z is -CONHN=CH-, and *m* is 0.1 to 10.

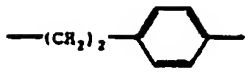
49. A conjugate according to Claim 39 wherein CH$_3$-SSS-W is LL-E33288α_3^I, Q is -CONHNH$_2$, Sp is -CH$_2$CH$_2$-, Y is -CHO, Z is -CONHNHCH$_2$-, and *m* is 0.1 to 10.

50. A conjugate according to Claim 33 wherein CH$_3$-SSS-W is N-acetyl LL-E33288γ_1^I, Q is hydroxysuccinimidocarbonyl, Sp is -CH$_2$CH$_2$-, Y is -NH$_2$, Z is -CONH-, and *m* is 0.5 to 15.

51. A conjugate according to Claim 34 wherein CH$_3$-SSS-W is N-acetyl LL-E33288γ_1^I, Q is hydroxysuccinimidocarbonyl, Sp is -CH$_2$CH(CH$_3$)-, Y is -NH$_2$, Z is -CONH-, and *m* is 0.5 to 15.

52. A conjugate according to Claim 36 wherein CH$_3$-SSS-W is N-acetyl LL-E33288γ_1^I Y, Q is hydroxysuccinimidocarbonyl, Sp is -CH$_2$C(CH$_3$)$_2$-, Y is -NH$_2$, Z is -CONH-, and *m* is 0.5 to 15.

53. A conjugate according to Claim 44 wherein CH3-SSS-W is N-acetyl LL-E33288γ_1^I, Q is -CONHNH$_2$, Sp is



Y is -CHO, Z is -CONNH=CH-, and *m* is 0.1 to 10.

54. A pharmaceutical composition comprising an antibody molecule according to any one of claims 1 to 19 or a conjugate molecule according to any one of claims 23 to 53 together with a pharmaceutically acceptable excipient, diluent or carrier.

Patentansprüche

1. Ein Antikörpermolekül mit Spezifität für Humanen Milchfett Globulus (HMFG), das eine zusammengesetzte schwere Kette und eine komplementäre leichte Kette umfaßt, wobei:

der variable Bereich dieser zusammengesetzten schweren Kette vorwiegend Rahmenregionreste eines humanen Immunglobulins oder eines Analogons desselben enthält;
mindestens die Aminosäurereste 2, 26 bis 35, 37, 50 bis 65, 71, 73, 95 bis 105 und 107 (nach dem Kabat-Numerierungssystem) in dem variablen Bereich der genannten schweren Kette von den entsprechenden Resten in dem monoklonalen Antikörper CTMO1 (vgl. Sequenz ID Nr. 1) stammen; und
die übrigen von Immunglobulin stammenden Teile der schweren Kette von einem humanen Immunglobulin oder einem Analogon desselben stammen.

2. Ein Antikörpermolekül mit Spezifität für Humanen Milchfett Globulus (HMFG), das eine zusammengesetzte schwere Kette und eine komplementäre leichte Kette umfaßt, wobei

der variable Bereich dieser zusammengesetzten schweren Kette vorwiegend Rahmenregionreste eines humanen Immunglobulins oder eines Analogons desselben enthält;
mindestens die Aminosäurereste 2, 26 bis 35, 37, 48, 50 bis 65, 67, 69, 71, 73, 95 bis 105 und 107 (nach dem Kabat-Numerierungssystem) in dem variablen Bereich der genannten schweren Kette von den entsprechenden Resten in dem monoklonalen Antikörper CTMO1 (vgl. Sequenz ID Nr. 1) stammen; und
die übrigen von Immunglobulin stammenden Teile der schweren Kette von einem humanen Immunglobulin oder einem Analogon desselben stammen.

3. Das Antikörpermolekül nach Anspruch 1 oder 2, worin zusätzlich in der genannten zusammengesetzten schweren Kette mindestens einer der Reste 6, 23, 49, 76, 78, 80, 88 und 91 von den entsprechenden Resten in dem monoklonalen Antikörper CTMO1 (vgl. Sequenz ID Nr. 1) stammt.

4. Das Antikörpermolekül nach einem der Ansprüche 1 bis 3, worin die komplementäre leichte Kette eine zusammengesetzte leichte Kette ist, in welcher:

der variable Bereich dieser zusammengesetzten leichten Kette vorwiegend Rahmenregionreste eines humanen Immunglobulins oder eines Analogons desselben enthält;
mindestens die Aminosäurereste 3, 24 bis 34, 36, 50 bis 56, 63, 91 bis 96 und 108 (nach dem Kabat-Numerierungssystem) in dem variablen Bereich der genannten leichten Kette von den entsprechenden Resten in dem monoklonalen Antikörper CTMO1 (vgl. Sequenz ID Nr. 2) stammen; und
die übrigen von Immunglobulin stammenden Teile der leichten Kette von einem humanen Immunglobulin oder einem Analogon desselben stammen.

5. Das Antikörpermolekül nach einem der Ansprüche 1 bis 3, worin die komplementäre leichte Kette eine zusammengesetzte leichte Kette ist, in welcher:

der variable Bereich dieser zusammengesetzten leichten Kette vorwiegend Rahmenregionreste eines humanen Immunglobulins oder eines Analogons desselben enthält;
mindestens die Aminosäurereste 3, 24 bis 34, 36, 37, 45, 48, 50 bis 56, 63, 91 bis 96 und 108 (nach dem Kabat-Numerierungssystem) in dem variablen Bereich der genannten leichten Kette von den entsprechenden

Resten in dem monoklonalen Antikörper CTMO1 (vgl. Sequenz ID Nr. 2) stammen; und
die übrigen von Immunglobulin stammenden Teile der leichten Kette von einem humanen Immunglobulin oder einem Analogon desselben stammen.

6. Ein Antikörpermolekül mit Spezifität für Humanen Milchfett Globulus (HMFG), das eine zusammengesetzte leichte Kette und eine komplementäre schwere Kette umfaßt, wobei:

der variable Bereich dieser zusammengesetzten leichten Kette vorwiegend Rahmenregionreste eines humanen Immunglobulins oder eines Analogons desselben enthält;
mindestens die Aminosäurereste 3, 24 bis 34, 36, 50 bis 56, 63, 91 bis 96 und 108 (nach dem Kabat-Numerierungssystem) in dem variablen Bereich der genannten leichten Kette von den entsprechenden Resten in dem monoklonalen Antikörper CTMO1 (vgl. Sequenz ID Nr. 2) stammen; und
die übrigen von Immunglobulin stammenden Teile der leichten Kette von einem humanen Immunglobulin oder einem Analogon desselben stammen.

7. Ein Antikörpermolekül mit Spezifität für Humanen Milchfett Globulus (HMFG), das eine zusammengesetzte leichte Kette und eine komplementäre schwere Kette umfaßt, wobei:

der variable Bereich dieser zusammengesetzten leichten Kette vorwiegend Rahmenregionreste eines humanen Immunglobulins oder eines Analogons desselben enthält;
mindestens die Aminosäurereste 3, 24 bis 34, 36, 37, 45, 48, 50 bis 56, 63, 91 bis 96 und 108 (nach dem Kabat-Numerierungssystem) in dem variablen Bereich der genannten leichten Kette von den entsprechenden Resten in dem monoklonalen Antikörper CTMO1 (vgl. Sequenz ID Nr. 2) stammen; und
die übrigen von Immunglobulin stammenden Teile der leichten Kette von einem humanen Immunglobulin oder einem Analogon desselben stammen.

8. Das Antikörpermolekül nach einem der Ansprüche 4 bis 7, worin zusätzlich in der genannten zusammengesetzten leichten Kette die Reste 89, 90 und 97 von den entsprechenden Resten in dem monoklonalen Antikörper CTMO1 (vgl. Sequenz ID Nr. 2) stammen.

9. Das Antikörpermolekül nach einem der Ansprüche 4 bis 8, worin zusätzlich in der genannten zusammengesetzten leichten Kette mindestens einer der Reste 1, 2, 49, 60, 70, 84, 85 und 87 von den entsprechenden Resten in dem monoklonalen Antikörper CTMO1 (vgl. Sequenz ID Nr. 2) stammt.

10. Das Antikörpermolekül nach einem der Ansprüche 1 bis 9, worin die humanen Rahmenreste in der dem variablen Bereich der zusammengesetzten leichten Kette und/oder in dem variablen Bereich der zusammengesetzten schweren Kette von den humanen variablen Bereichssequenzen LAY, POM, TUR, TEI, KOL, NEWM, REI oder EU stammen.

11. Das Antikörpermolekül nach Anspruch 10, worin die humanen Rahmenreste von der humanen EU Sequenz stammen.

12. Das Antikörpermolekül nach einem der Ansprüche 1 bis 11, das ein komplettes Immunglobulin ist.

13. Das Antikörpermolekül nach Anspruch 12, worin die konstante Region der schweren Kette eine der humanen IgG Unterklasse ist.

14. Das Antikörpermolekül nach Anspruch 13, worin die konstante Region der schweren Kette eine der humanen IgG4 Unterklasse ist.

15. Das Antikörpermolekül nach nach Anspruch 13, worin die konstante Region der schweren Kette eine der humanen IgG4 Unterklasse mit einem Prolinrest an der Position 241 ist.

16. Das Antikörpermolekül nach einem der Ansprüche 1 bis 11, das ein Fab-, Fab'- oder $F(ab')_2$-Fragment ist.

17. Das Antikörpermolekül nach einem der Ansprüche 12 bis 16, worin der konstante Bereich der leichten Kette einer der humanen kappa-Klasse ist.

18. Das Antikörpermolekül nach einem der Ansprüche 1 bis 11, das ein Fv- oder ein Einzelketten-Fv-Fragment ist.

19. Das Antikörpermolekül nach nach einem der Ansprüche 1 bis 18, das durch rekombinante DNA-Technologie hergestellt ist.

20. Ein Verfahren zur Herstellung des Antikörpermoleküls nach einem der Ansprüche 1 bis 19, welches Verfahren umfaßt:

(a) in einem Expressionsvektor die Herstellung eines Operons mit einer DNA-Sequenz, die für die schwere Kette eines Antikörpermoleküls gemäß der Definition in einem der Ansprüche 1 bis 19 codiert;
(b) in einem Expressionsvektor die Herstellung eines Operons mit einer DNA-Sequenz, die für die leichte Kette eines Antikörpermoleküls gemäß der Definition in einem der Ansprüche 1 bis 19 codiert;
(c) Transfektion einer Wirtszelle mit dem oder mit jedem Vektor und
(d) Züchtung der transfizierten Zell-Linie zur Herstellung des Antikörpermoleküls.

21. Das Verfahren nach Anspruch 20 zur Herstellung eines Antikörper-Fragmentes nach Anspruch 16 oder 18, in welchem die Wirtszelle eine bakterielle Wirtszelle ist.

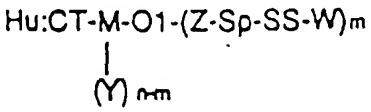
22. Das Verfahren nach Anspruch 20, in welchem die Wirtszelle eine Säugetier-Wirtszelle ist.

23. Ein Konjugatmolekül, welches das Antikörpermolekül nach einem der Ansprüche 1 bis 19 an ein Effektormolekül oder an ein Reportermolekül konjugiert umfaßt.

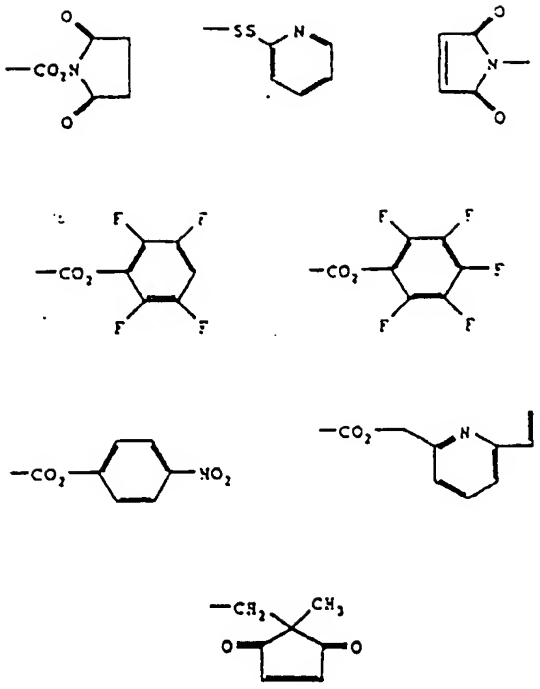
24. Ein Konjugatmolekül nach Anspruch 23, in welchem das Effektormolekül ein Methyltrithio-Antitumoragens ist.

25. Ein Konjugatmolekül nach Anspruch 24, in welchem das Methyltrithio-Antitumoragens ein Disulfid-Analogon der α_1-, α_2-, α_3-, α_4-, β_1-, β_2-, γ_1-, δ_1- und Pseudoaglycon-Komponenten des Komplexes LL-E33288 und von Derivaten desselben, der Antitumor-Antibiotika BBM-1675, FR-900405, FR-900406, PD 114759, PD 115028, CL-1577A, CL-1577B, CL-1577D, CL-1577E und CL 1724 und von Derivaten derselben ist.

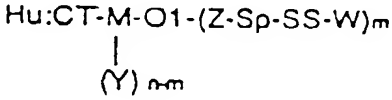
26. Ein Konjugatmolekül nach Anspruch 25 der Formel



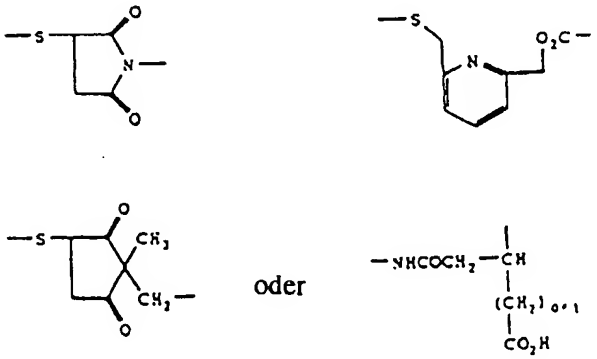
hergestellt aus einer Verbindung der Formel CH_3-SSS-W, worin CH_3-SSS-W ein Antitumor-Antibiotikum mit der Bezeichnung LL-E33288α_1^{Br}, α_1^{I}-, α_2^{Br}-, α_3^{Br}-, α_3^{I}-, α_4^{Br}-, β_1^{Br}-, β_1^{I}, β_2^{Br}, β_2^{I}-, γ_1^{Br}-, γ_1^{I}-, δ_1^{I}, Iod- oder Brom-Pseudoaglycon, deren Dihydro- oder N-Acyl-Gegenstücken, BBM-1675, FR-900405, FR-900406, PD 114759, PD 115028, CL-1577A, CL-1577B, CL-1577D, CL-1577E, CL 1724 oder deren N-Acetyl-Gegenstücken ist, umfassend:
die Umsetzung von CH_3-SSS-W mit einer Verbindung der allgemeinen Formel Q-Sp-SH, worin Sp ein geradkettiger oder verzweigter zweiwertiger oder dreiwertiger (C_1-C_{18})-Rest, ein zweiwertiger oder dreiwertiger Aryl- oder Heteroarylrest, ein zweiwertiger oder dreiwertiger (C_3-C_{18})-Cycloalkyl- oder -Heterocycloalkylrest, ein zweiwertiger oder dreiwertiger Aryl- oder Heteroaryl-(C_1-C_{18})-alkylrest, ein zweiwertiger oder dreiwertiger Cycloalkyl- oder Heterocycloalkyl-(C_1-C_{18})-alkylrest oder ein zweiwertiger oder dreiwertiger ungesättigter (C_2-C_{18})-Alkylrest ist, worin, wenn Sp ein dreiwertiger Rest ist, dieser zusätzlich durch Amino-, Alkylamino-, Arylamino-, Heteroarylamino-, Carboxyl-, Niedrigalkoxy-, Hydroxy-, Thiol- oder Niedrigalkylthiogruppen substituiert sein kann; und Q eine der folgenden Gruppen ist oder anschließend in diese umgewandelt werden kann: Halogen, Amino, Alkylamino, Carboxyl, Carboxaldehyd, Hydroxy, Thiol, a-Haloacetyloxy, Niedrigalkyldicarboxyl, -$CONHNH_2$, -$NHCONHNH_2$, -$NHCSNHNH_2$, -ONH_2, -$CONH_3$,



zur Herstellung eines Zwischenprodukts der Formel Q-Sp-SS-W, worin Q, Sp und W die oben genannte Bedeutung haben,
die Umsetzung von Q-Sp-SS-W mit einem Molekül der Formel Hu:CT-M-01-$(Y)_n$, worin Hu:CT-M-01 ein HAM gemäß den Ansprüchen 1 bis 17 und Y eine Seitenkettenamino-, -carboxyl- oder -thiolgruppe eines Proteins, ein von Glycoprotein-Kohlenhydratresten abgeleiterer Aldehyd oder eine Amidoalkylthiogruppe ist und n eine ganze Zahl von 1 bis 100 bedeutet, zur Herstellung einer Verbindung der Formel:

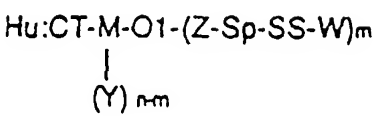


worin Y, Sp, W und n die oben genannte Bedeutung haben und Z durch kovalente Reaktion der Gruppen Q und Y direkt oder nach anschließender Reduktion gebildet ist und Z für -CONH-, -CONHN=CH-, -CONHNHCH$_2$-, -NHC-SNHN=CH-, -NHCH$_2$-, -N=CH-, -CO$_2$-, -NHCH$_2$CO$_2$-, -SS-,

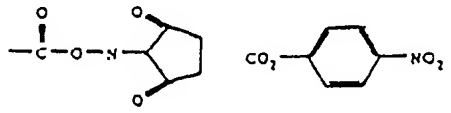


und m für 0,1 bis 15 steht.

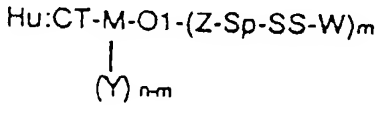
27. Ein Konjugat nach Anspruch 26 der Formel



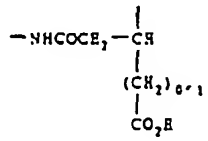
hergestellt aus der Klasse der Antitumor-Antibiotika mit der Bezeichnung LL-E33288 (CH_3-SSS-W), umfassend: die Verdrängung der Dithiomethylgruppierung mit einer Verbindung der Formel Q-Sp-SH, worin Sp für geradkettige oder verzweigtkettige zweiwertige oder dreiwertige (C_2-C_{10})-Reste oder zweiwertige oder dreiwertige Aryl- oder -Heteroaryl-(C_2-C_5)-alkylreste steht, worin Sp, wenn es ein dreiwertiger Rest ist, zusätzlich durch Amino-, Heteroarylamino-, Hydroxy- oder Thiolgruppen substituiert sein kann und Q für folgende Gruppen steht oder anschließend in sie umgewandelt werden kann: Carboxyl, Niedrigalkyldicarboxylanhydrid, -CONHNH$_2$ oder



zur Herstellung einer Zwischenverbindung der allgemeinen Formel Q-Sp-SS-W, worin Q, Sp und W die oben genannte Bedeutung haben,
die Umsetzung von Q-Sp-SS-W mit einem Molekül der Formel Hu:CT-M-O1-(Y)$_n$, worin Y eine Seitenkettenaminogruppe an dem Antikörper oder ein durch Oxidation der Kohlenhydratgruppen des Antikörpers gebildeter Aldehyd ist und n für eine ganze Zahl von 1 bis 100 steht, zur Herstellung einer Verbindung der Formel



worin Y, Sp, W und n die oben genannte Bedeutung haben und Z durch eine kovalente Reaktion der Gruppen Q und Y direkt oder nach anschließender Reduktion gebildet ist und Z für -CONH-, -CONHN=CH-, -CONHNHCH$_2$- oder



und m für 0,1 bis 15 steht.

28. Ein Konjugat nach Anspruch 27, worin CH_3-SSS-W das Antitumor-Antibiotikum mit der Bezeichnung LL-E33288γ_1^I ist.

29. Ein Konjugat nach Anspruch 27, worin CH_3-SSS-W das Antitumor-Antibiotikum mit der Bezeichnung LL-E33288α_2^I ist.

30. Ein Konjugat nach Anspruch 27, worin CH_3-SSS-W das Antitumor-Antibiotikum mit der Bezeichnung LL-E33288α_3^I ist.

31. Ein Konjugat nach Anspruch 27, worin CH_3-SSS-W das Antitumor-Antibiotikum mit der Bezeichnung N-Acetyl-LL-

E33288γ_1^I ist.

32. Ein Konjugat nach Anspruch 27, worin CH_3-SSS-W das Antitumor-Antibiotikum mit der Bezeichnung Iod-LL-E33288-Pseudoaglycon ist.

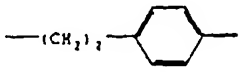
33. Ein Konjugat nach Anspruch 27, worin Q für den Hydroxysuccinimidester einer Carboxylgruppe, Sp für $-CH_2CH_2-$, Y für $-NH_2$, Z für -CONH- und m für 0,5 bis 15 steht.

34. Ein Konjugat nach Anspruch 27, worin Q für den Hydroxysuccinimidester einer Carboxylgruppe, Sp für $-CH_2CH(CH_3)-$, Y für $-NH_2$, Z für -CONH- und m für 0,5 bis 15 steht.

35. Ein Konjugat nach Anspruch 27, worin Q für den 4-Nitrophenylester einer Carboxylgruppe, Sp für $-CH_2CH_2-$, Y für $-NH_2$, Z für -CONH- und m für 0,5 bis 15 steht.

36. Ein Konjugat nach Anspruch 27, worin Q für den Hydroxysuccinimidester einer Carboxylgruppe, Sp für $-CH_2C(CH_3)_2-$, Y für $-NH_2$, Z für -CONH- und m für 0,5 bis 15 steht.

37. Ein Konjugat nach Anspruch 27, worin Q für den Hydroxysuccinimidester einer Carboxylgruppe, Sp für



Y für NH_2, Z für -CONH- und m für 0,5 bis 15 steht.

38. Ein Konjugat nach Anspruch 27, worin Q für $-CONHNH_2$, Sp für $-CH_2CH_2-$, Y für -CHO, Z für -CONHN=CH- und m für 0,1 bis 10 steht.

39. Ein Konjugat nach Anspruch 27, worin Q für $-CONHNH_2$, Sp für $-CH_2CH_2-$, Y für -CHO, Z für $-CONHNHCH_2-$ und m für 0,1 bis 10 steht.

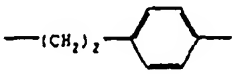
40. Ein Konjugat nach Anspruch 27, worin Q für $-CONHNH_2$, Sp für $-CH_2CH(CH_3)-$, Y für -CHO, Z für -CONHN=CH- und m für 0,1 bis 10 steht.

41. Ein Konjugat nach Anspruch 27, worin Q für $-CONHNH_2$, Sp für $-CH_2CH(CH_3)-$, Y für -CHO, Z für $-CONHNHCH_2-$ und m für 0,1 bis 10 steht.

42. Ein Konjugat nach Anspruch 27, worin Q für $-CONHNH_2$, Sp für $-CH_2C(CH_3)_2-$, Y für -CHO, Z für -CONHN=CH- und m für 0,1 bis 10 steht.

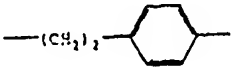
43. Ein Konjugat nach Anspruch 27, worin Q für $-CONHNH_2$, Sp für $-CH_2C(CH_3)_2-$, Y für -CHO, Z für $-CONHNHCH_2-$ und m für 0,1 bis 10 steht.

44. Ein Konjugat nach Anspruch 27, worin Q für $-CONHNH_2$, Sp für



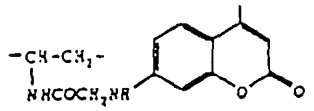
Y für -CHO, Z für -CONHN=CH- und m für 0,1 bis 10 steht.

45. Ein Konjugat nach Anspruch 27, worin Q für $-CONHNH_2$, Sp für



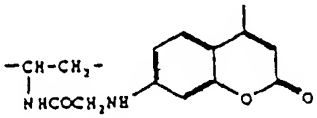
Y für -CHO, Z für $-CONHNHCH_2-$ und m für 0,1 bis 10 steht.

46. Ein Konjugat nach Anspruch 27, worin Q für -CONHNH$_2$, Sp für



Y für -CHO, Z für -CONHN=CH- und m für 0,1 bis 10 steht.

47. Ein Konjugat nach Anspruch 27, worin Q für -CONHNH$_2$, Sp für



Y für -CHO, Z für -CONHNHCH$_2$- und m für 0,1 bis 10 steht.

48. Ein Konjugat nach Anspruch 38, worin CH_3-SSS-W für LL-E33288γ_1^I, Q für -CONHNH$_2$, Sp für -CH_2CH_2-, Y für -CHO, Z für -CONHN=CH- und m für 0,1 bis 10 steht.

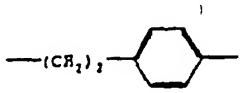
49. Ein Konjugat nach Anspruch 39, worin CH_3-SSS-W für LL-E33288α_3^I, Q für -CONHNH$_2$, Sp für -CH_2CH_2-, Y für -CHO, Z für -CONHNHCH$_2$- und m für 0,1 bis 10 steht.

50. Ein Konjugat nach Anspruch 33, worin CH_3-SSS-W für N-Acetyl-LL-E33288γ_1^I, Q für Hydroxysuccinimidocarbonyl, Sp für -CH_2CH_2-, Y für -NH$_2$, Z für -CONH- und m für 0,5 bis 15 steht.

51. Ein Konjugat nach Anspruch 34, worin CH_3-SSS-W für N-Acetyl-LL-E33288γ_1^I, Q für Hydroxysuccinimidocarbonyl, Sp für -$CH_2CH(CH_3)$-, Y für -NH$_2$, Z für -CONH- und m für 0,5 bis 15 steht.

52. Ein Konjugat nach Anspruch 36, worin CH_3-SSS-W für N-Acetyl-LL-E33288γ_1^I, Q für Hydroxysuccinimodocarbonyl, Sp für -$CH_2C(CH_3)_2$-, Y für -NH$_2$, Z für -CONH- und m für 0,5 bis 15 steht.

53. Ein Konjugat nach Anspruch 44, worin CH_3-SSS-W für N-Acetyl-LL-E33288γ_1^I, Q für -CONHNH$_2$, Sp für



Y für -CHO, Z für -CONNH-CH- und m für 0,1 bis 10 steht.

54. Eine pharmazeutische Zusammensetzung, die ein Antikörpermolekül nach einem der Ansprüche 1 bis 19 oder ein Konjugatmolekül nach einem der Ansprüche 23 bis 53 gemeinsam mit einem pharmazeutisch verwendbaren Exzipienten, Verdünnungsmittel oder Träger enthält.

Revendications

1. Molécule d'anticorps ayant une spécificité pour le globule de matière grasse de lait humain (HMFG), comprenant une chaîne lourde composite et une chaîne légère complémentaire, dans laquelle:

le domaine variable de ladite chaîne lourde composite comprend principalement des résidus de la région charpente d'une immunoglobuline humaine ou d'un de ses analogues;
les résidus d'acides aminés 2, 26 à 35, 37, 50 à 65, 71, 73, 95 à 105 et 107 (selon le système de numérotation de Kabat) au moins, dans ledit domaine variable de chaîne lourde, sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme le montre la séquence ID N° 1); et

les parties restantes dérivées d'immunoglobuline de la chaîne lourde sont dérivées d'une immunoglobuline humaine ou d'un de ses analogues.

2. Molécule d'anticorps ayant une spécificité pour le globule de matière grasse de lait humain (HMFG), comprenant une chaîne lourde composite et une chaîne légère complémentaire, dans laquelle:

le domaine variable de ladite chaîne lourde composite comprend principalement des résidus de la région charpente d'une immunoglobuline humaine ou d'un de ses analogues;
les résidus d'acides aminés 2, 26 à 35, 37, 48, 50 à 65, 67, 69, 71, 73, 95 à 105 et 107 (selon le système de numérotation de Kabat) au moins, dans ledit domaine variable de chaîne lourde, sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMOl (comme le montre la séquence ID N° 1); et
les parties restantes dérivées d'immunoglobuline de la chaîne lourde sont dérivées d'une immunoglobuline humaine ou d'un de ses analogues.

3. Molécule d'anticorps selon la revendication 1 ou la revendication 2, dans laquelle en outre, dans ladite chaîne lourde composite, au moins un des résidus 6, 23, 49, 76, 78, 80, 88 et 91 est dérivé du résidu correspondant dans l'anticorps monoclonal CTMOl (comme le montre la séquence ID N° 1).

4. Molécule d'anticorps selon l'une quelconque des revendications 1 à 3, dans laquelle la chaîne légère complémentaire est une chaîne légère composite dans laquelle:

le domaine variable de ladite chaîne légère composite comprend principalement des résidus de la région charpente d'une immunoglobuline humaine ou d'un de ses analogues;
les résidus d'acides aminés 3, 24 à 34, 36, 50 à 56, 63, 91 à 96 et 108 (selon le système de numérotation de Kabat) au moins, dans ledit domaine variable de chaîne légère, sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme le montre la séquence ID N° 2); et
les parties restantes dérivées d'immunoglobuline de la chaîne légère sont dérivées d'une immunoglobuline humaine ou d'un de ses analogues.

5. Molécule d'anticorps selon l'une quelconque des revendications 1 à 3, dans laquelle la chaîne légère complémentaire est une chaîne légère composite dans laquelle:

le domaine variable de ladite chaîne légère composite comprend principalement des résidus de la région charpente d'une immunoglohuline humaine ou d'un de ses analogues;
les résidus d'acides aminés 3, 24 à 34, 36, 37, 45, 48, 50 à 56, 63, 91 à 96 et 108 (selon le système de numérotation de Kabat) au moins, dans ledit domaine variable de chaîne légère, sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme le montre la séquence ID N° 2); et
les parties restantes dérivées d'immunoglobuline de la chaîne légère sont dérivées d'une immunoglobuline humaine ou d'un de ses analogues.

6. Molécule d'anticorps ayant une spécificité pour le globule de matière grasse de lait humain (HMFG), comprenant une chaîne légère composite et une chaîne lourde complémentaire, dans laquelle:

le domaine variable de ladite chaîne légère composite comprend principalement des résidus de la région charpente d'une immunoglobuline humaine ou d'un de ses analogues;
les résidus d'acides aminés 3, 24 à 34, 36, 50 à 56, 63, 91 à 96 et 108 (selon le système de numérotation de Kabat) au moins, dans ledit domaine variable de chaîne légère, sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme le montre la séquence ID N° 2); et
les parties restantes dérivées d'immunoglobuline de la chaîne légère sont dérivées d'une immunoglobuline humaine ou d'un de ses analogues.

7. Molécule d'anticorps ayant une spécificité pour le globule de matière grasse de lait humain (HMFG), comprenant une chaîne légère composite et une chaîne lourde complémentaire, dans laquelle:

le domaine variable de ladite chaîne légère composite comprend principalement des résidus de la région charpente d'une immunoglobuline humaine ou d'un de ses analogues;
les résidus d'acides aminés 3, 24 à 34, 36, 37, 45, 48, 50 à 56, 63, 91 à 96 et 108 (selon le système de numérotation de Kabat) au moins, dans ledit domaine variable de chaîne légère, sont dérivés des résidus

correspondants dans l'anticorps monoclonal CTMO1 (comme le montre la séquence ID N° 2); et
les parties restantes dérivées d'immunoglobuline de la chaîne légère sont dérivées d'une immunoglobuline humaine ou d'un de ses analogues.

8. Molécule d'anticorps selon l'une quelconque des revendications 4 à 7, dans laquelle en outre, dans ladite chaîne légère composite, les résidus 89, 90 et 97 sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme le montre la séquence ID N°2).

9. Molécule d'anticorps selon l'une quelconque des revendications 4 à 8, dans laquelle en outre, dans ladite chaîne légère composite, au moins un des résidus 1, 2, 49, 60, 70, 84, 85 et 87, est dérivé du résidu correspondant dans l'anticorps monoclonal CTMOI (comme le montre la séquence ID N° 2).

10. Molécule d'anticorps selon l'une quelconque des revendications 1 à 9, dans laquelle les résidus de charpente humaine dans le domaine variable de la chaîne légère composite et/ou dans le domaine variable de la chaîne lourde composite sont dérivés des séquences de domaine variable de LAY, POM, TUR, TEI, KOL, NEWM, REI ou EU humain.

11. Molécule d'anticorps selon la revendication 10, dans laquelle les résidus de charpente humaine sont dérivés de la séquence de EU humain.

12. Molécule d'anticorps selon l'une quelconque des revendications 1 à 11, qui est une immunoglobuline complète.

13. Molécule d'anticorps selon la revendication 12, dans laquelle la région constante de la chaîne lourde est celle de la classe des IgG humaines.

14. Molécule d'anticorps selon la revendication 13, dans laquelle la région constante de la chaîne lourde est celle de la sous-classe d'IgG4 humaine.

15. Molécule d'anticorps selon la revendication 13, dans laquelle la région constante de la chaîne lourde est celle de la sous-classe d'IgG4 humaine avec un résidu proline en position 241.

16. Molécule d'anticorps selon l'une quelconque des revendications 1 à 11, qui est un fragment Fab, Fab' ou $F(ab')_2$.

17. Molécule d'anticorps selon l'une quelconque des revendications 12 à 16, dans laquelle le domaine constant de la chaîne légère est celui de la classe kappa humaine.

18. Molécule d'anticorps selon l'une quelconque des revendications 1 à 11, qui est un fragment Fv ou un fragment Fv à une seule chaîne.

19. Molécule d'anticorps selon l'une quelconque des revendications 1 à 18, qui est produite par la technologie de recombinaison d'ADN.

20. Procédé pour produire la molécule d'anticorps selon l'une quelconque des revendications 1 à 19, ledit procédé comprenant les étapes qui consistent à:

(a) produire dans un vecteur d'expression, un opéron ayant une séquence d'ADN qui code pour une chaîne lourde de molécule d'anticorps telle que définie dans l'une quelconque des revendications 1 à 19;
(b) produire dans un vecteur d'expression, un opéron ayant une séquence d'ADN qui code pour une chaîne légère de molécule d'anticorps telle que définie dans l'une quelconque des revendications 1 à 19;
(c) transfecter une cellule hôte avec le vecteur ou avec chaque vecteur; et
(d) cultiver la lignée cellulaire transfectée pour produire la molécule d'anticorps.

21. Procédé selon la revendication 20, pour la production d'un fragment d'anticorps selon la revendication 16 ou la revendication 18, dans lequel la cellule hôte est une cellule hôte bactérienne.

22. Procédé selon la revendication 20, dans lequel la cellule hôte est une cellule hôte de mammifère.

23. Molécule conjuguée comprenant la molécule d'anticorps selon l'une quelconque des revendications 1 à 19, con-

juguée à une molécule d'effecteur ou à une molécule de reporteur.

24. Molécule conjuguée selon la revendication 23, dans laquelle la molécule d'effecteur est un agent antitumoral de type méthyltrithio.

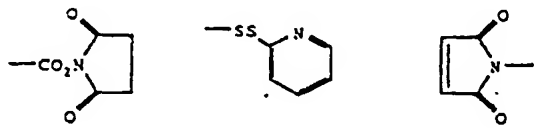
25. Molécule conjuguée selon la revendication 24, dans laquelle l'agent antitumoral de type méthyltrithio est un analogue bisulfure des composants α_1, α_2, α_3, α_4, β_1, β_2, γ_1, δ_1 et pseudoaglycone du complexe LL-E33288 et de ses dérivés, des antibiotiques antitumoraux BBM-1675, FR-900405, FR-900406, PD 114759, PD 115028, CL-1577A, CL-1577B, CL-1577D, CL-1577E et CL 1724 et de leurs dérivés.

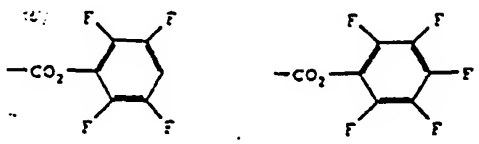
26. Molécule conjuguée selon la revendication 25, de fonnule

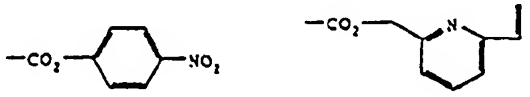
$$\mathrm{Hu{:}CT\text{-}M\text{-}O1\text{-}(Z\text{-}Sp\text{-}SS\text{-}W)_m}$$
$$\mathrm{|}$$
$$\mathrm{(Y)_{n-m}}$$

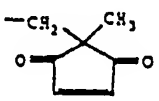
préparée à partir d'un composé de formule CH_3-SSS-W, où CH_3-SSS-W est un antibiotique antitumoral désigné par LL-E33288α_1^{Br}, α_1^{I}, α_2^{Br}, α_3^{Br}, α_3^{I}, α_4^{Br}, β_1^{Br}, β_1^{I}, β_2^{Br}, β_2^{I}, γ_1^{Br}, γ_1^{I}, δ_1^{I}, l'iodo- ou le bromopseudoaglycone, leurs homologues dihydro ou N-acyl, BBM-1675, FR-900405, FR-900406, PD 114759, PD 115028, CL-1577A, CL-1577B, CL-1577D, CL-1577E, CL 1724 ou leurs homologues N-acétyl:

en faisant réagir CH_3-SSS-W avec un composé de formule générale Q-Sp-SH, dans laquelle Sp est un radical (en C_1-C_{18}) divalent ou trivalent à chaîne linéaire ou ramifiée, un radical aryle ou hétéroaryle divalent ou trivalent, un radical cycloalkyle ou hétérocycloalkyle (en C_3-C_{18}) divalent ou trivalent, un radical aiyl- ou hétéroalyl-alkyle (en C_1-C_{18}) divalent ou trivalent, un radical cycloalkyl- ou hétérocycloalkyl-alkyle (en C_1-C_{18}) divalent ou trivalent ou un radical alkyle insaturé (en C_2-C_{18}) divalent ou trivalent, dans laquelle, si Sp est un radical trivalent, il peut être en outre substitué par des groupes amino, alkylamino, arylamino, hétéroalylamino, carboxyle, alcoxy inférieur, hydroxy, thiol, ou alkylthio inférieur; et Q est converti, ou peut être converti par la suite, en groupe halogéno, amino, alkylamino, carboxyle, carboxaldéhyde, hydroxy, thiol, a-halogénoacétyloxy, alkyl(inférieur)dicarboxyle, -$CONHNH_2$, -$NHCONHNH_2$, -$NHCSNHNH_2$, -ONH_2, -CON_3,







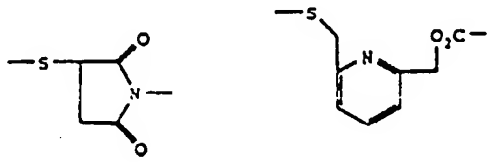


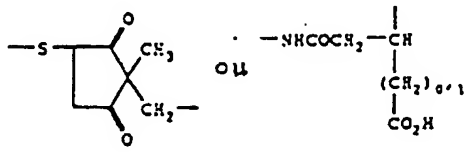
pour produire un intermédiaire de formule Q-Sp-SS-W, dans laquelle Q, Sp, et W sont tels que définis ci-dessus,

en faisant réagir Q-Sp-SS-W avec une molécule de formule Hu:CT-M-O1-$(Y)_n$ dans laquelle Hu:CT-M-O1 est une HAM selon les revendications 1 à 17 et Y est un groupe amino, carboxyle, ou thiol de chaîne latérale d'une protéine, un aldéhyde dérivé des résidus glucidiques de glycoprotéines ou un groupe amidoalkylthio; et n est un nombre entier de 1 à 100, pour produire un composé de formule:

$$\mathrm{Hu{:}CT\text{-}M\text{-}O1\text{-}(Z\text{-}Sp\text{-}SS\text{-}W)_m}$$
$$\mathrm{(Y)_{n-m}}$$

dans laquelle Y, Sp, W et n sont tels que définis ci-dessus, et Z est formé à partir d'une réaction covalente des groupes Q et Y, directement ou après une réduction ultérieure, et Z est -CONH-, -CONHN=CH-, $-CONHNHCH_2-$, -NHCSNHN=CH-, $-NHCH_2-$, -N=CH-, $-CO_2-$, $-NHCH_2CO_2-$, -SS-,





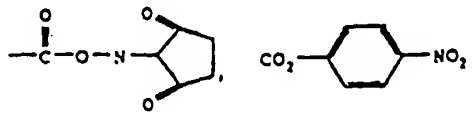
et m est égal à une valeur allant de 0,1 à 15.

27. Conjugué selon la revendication 26, de formule :

$$\mathrm{Hu{:}CT\text{-}M\text{-}O1\text{-}(Z\text{-}Sp\text{-}SS\text{-}W)_m}$$
$$\mathrm{(Y)_{n-m}}$$

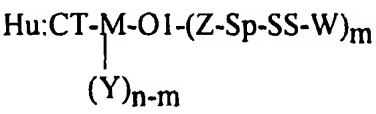
préparé à partir de la classe d'antibiotiques antitumoraux désignés par LL-E33288 (CH_3-SSS-W):

en déplaçant le groupement dithiométhyle avec un composé de formule Q-Sp-SH, dans laquelle Sp représente des radicaux (en C_2-C_{10}) divalents ou trivalents à chaîne linéaire ou ramifiée ou des radicaux aryl- ou hétéroaryl-(alkyle en C_2-C_5) divalents ou trivalents, dans laquelle si Sp est un radical trivalent, il peut être en outre substitué par des groupes amino, hétéroarylamino, hydroxy, ou thiols; et Q est converti, ou peut être converti par la suite, en groupe carboxyle, anhydride d'alkyl(inférieur)dicarboxyle, $-CONHNH_2$, ou

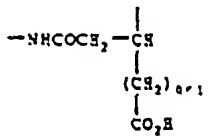


pour produire un intermédiaire de formule générale Q-Sp-SS-W, dans laquelle Q, Sp et W sont tels que définis ci-dessus,

en faisant réagir Q-Sp-SS-W avec une molécule de formule Hu:CT-M-O1-$(Y)_n$ dans laquelle Y est un groupe amino de chaîne latérale sur l'anticorps, ou un aldéhyde produit par oxydation des groupes glucidiques de l'anticorps, et n est un nombre entier de 1 à 100, pour produire un composé de formule:



dans laquelle Y, Sp, W et n sont tels que définis ci-dessus, et Z est formé à partir d'une réaction covalente des groupes Q et Y, directement ou après une réduction ultérieure, et Z est -CONH-, -CONHN=CH-, $-CONHNHCH_2-$ ou



et m est égal à une valeur allant de 0,1 à 15.

28. Conjugué selon la revendication 27, dans lequel CH_3-SSS-W est l'antibiotique antitumoral désigné par LL-E33288 γ_1^I.

29. Conjugué selon la revendication 27, dans lequel CH_3-SSS-W est l'antibiotique antitumoral désigné par LL-E33288 α_2^I.

30. Conjugué selon la revendication 27, dans lequel CH_3-SSS-W est l'antibiotique antitumoral désigné par LL-E33288 α_3^I.

31. Conjugué selon la revendication 27, dans lequel CH_3-SSS-W est l'antibiotique antitumoral désigné par N-acétyl LL-E33288 γ_1^I.

32. Conjugué selon la revendication 27, dans lequel CH_3-SSS-W est l'antibiotique antitumoral désigné par iodo LL-E33288 pseudoaglycone.

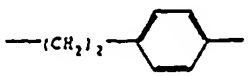
33. Conjugué selon la revendication 27, dans lequel Q est l'ester hydroxysuccinimide d'un groupe carboxyle, Sp est $-CH_2CH_2-$, Y est $-NH_2$, Z est -CONH-, et m est égal à 0,5 à 15.

34. Conjugué selon la revendication 27, dans lequel Q est l'ester hydroxysuccinimide d'un groupe carboxyle, Sp est $-CH_2CH(CH_3)-$, Y est $-NH_2$, Z est -CONH-, et m est égal à une valeur allant de 0,5 à 15.

35. Conjugué selon la revendication 27, dans lequel Q est l'ester 4-nitrophénylique d'un groupe carboxyle, Sp est $-CH_2CH_2-$, Y est $-NH_2$, Z est -CONH-, et m est égal à une valeur allant de 0,5 à 15.

36. Conjugué selon la revendication 27, dans lequel Q est l'ester hydroxysuccinimide d'un groupe carboxyle, Sp est $-CH_2C(CH_3)_2-$, Y est $-NH_2$, Z est -CONH-, et m est égal à une valeur allant de 0,5 à 15.

37. Conjugué selon la revendication 27, dans lequel Q est l'ester hydroxysuccinimide d'un groupe carboxyle, Sp est



Y est $-NH_2$, Z est -CONH-, et m est égal à une valeur allant de 0,5 à 15.

38. Conjugué selon la revendication 27, dans lequel Q est $-CONHNH_2$, Sp est $-CH_2CH_2-$, Y est -CHO, Z est -CONHN=CH-, et m est égal à une valeur allant de 0,1 à 10.

39. Conjugué selon la revendication 27, dans lequel Q est $-CONHNH_2$, Sp est $-CH_2CH_2-$, Y est -CHO, Z est $-CONHNHCH_2-$, et m est égal à une valeur allant de 0,1 à 10.

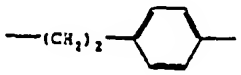
40. Conjugué selon la revendication 27, dans lequel Q est $-CONHNH_2$, Sp est $-CH_2CH(CH_3)-$, Y est -CHO, Z est -CONHN=CH-, et m est égal à une valeur allant de 0,1 à 10.

41. Conjugué selon la revendication 27, dans lequel Q est $-CONHNH_2$, Sp est $-CH_2CH(CH_3)-$, Y est -CHO, Z est $-CONHNHCH_2-$, et m est égal à une valeur allant de 0,1 à 10.

42. Conjugué selon la revendication 27, dans lequel Q est $-CONHNH_2$, Sp est $-CH_2C(CH_3)_2-$, Y est -CHO, Z est -CONHN=CH-, et m est égal à une valeur allant de 0,1 à 10.

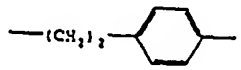
43. Conjugué selon la revendication 27, dans lequel Q est $-CONHNH_2$, Sp est $-CH_2C(CH_3)_2-$, Y est -CHO, Z est $-CONHNHCH_2-$, et m est égal à une valeur allant de 0,1 à 10.

44. Conjugué selon la revendication 27, dans lequel Q est $-CONHNH_2$, Sp est



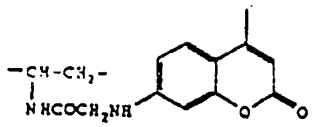
Y est -CHO, Z est -CONHN=CH-, et m est égal à une valeur allant de 0,1 à 10.

45. Conjugué selon la revendication 27, dans lequel Q est $-CONHNH_2$, Sp est



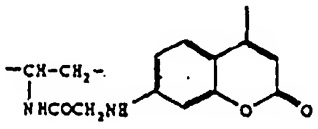
Y est -CHO, Z est $-CONHNHCH_2-$, et m est égal à une valeur allant de 0,1 à 10.

46. Conjugué selon la revendication 27, dans lequel Q est $-CONHNH_2$, Sp est



Y est -CHO, Z est -CONHN=CH-, et m est égal à une valeur allant de 0,1 à 10.

47. Conjugué selon la revendication 27, dans lequel Q est $-CONHNH_2$, Sp est



Y est -CHO, Z est $-CONHNHCH_2-$, et m est égal à une valeur allant de 0,1 à 10.

48. Conjugué selon la revendication 38, dans lequel CH_3-SSS-W est LL-E33288γ_1^I, Q est $-CONHNH_2$, Sp est

$-CH_2CH_2-$, Y est -CHO, Z est -CONHN=CH-, et m est égal à une valeur allant de 0,1 à 10.

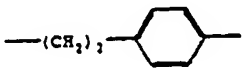
49. Conjugué selon la revendication 39, dans lequel CH_3-SSS-W est LL-E33288α_3^I, Q est $-CONHNH_2$, Sp est $-CH_2CH_2-$, Y est -CHO, Z est $-CONHNHCH_2-$, et m est égal à une valeur allant de 0,1 à 10.

50. Conjugué selon la revendication 33, dans lequel CH_3-SSS-W est N-acétyl LL-E33288γ_1^I, Q est un hydroxysuccinimidocarbonyle, Sp est $-CH_2-CH_2-$, Y est $-NH_2$, Z est -CONH-, et m est égal à une valeur allant de 0,5 à 15.

51. Conjugué selon la revendication 34, dans lequel CH_3-SSS-W est N-acétyl LL-E33288γ_1^I, Q est un hydroxysuccinimidocarbonyle, Sp est $-CH_2CH(CH_3)-$, Y est $-NH_2$, Z est -CONH-, et m est égal à une valeur allant de 0,5 à 15.

52. Conjugué selon la revendication 36, dans lequel CH_3-SSS-W est N-acétyl LL-E33288γ_1^I Y, Q est un hydroxysuccinimidocarbonyle, Sp est $-CH_2C(CH_3)_2-$, Y est $-NH_2$, Z est -CONH-, et m est égal à une valeur allant de 0,5 à 15.

53. Conjugué selon la revendication 44, dans lequel CH_3-SSS-W est N-acétyl LL-E33288γ_1^I, Q est $-CONHNH_2$, Sp est



Y est -CHO, Z est -CONHN=CH-, et m est égal à une valeur allant de 0,1 à 10.

54. Composition pharmaceutique comprenant une molécule d'anticorps selon l'une quelconque des revendications 1 à 19 ou une molécule conjuguée selon l'une quelconque des revendications 23 à 53, ainsi qu'un excipient, diluant ou véhicule acceptable d'un point de vue pharmaceutique.

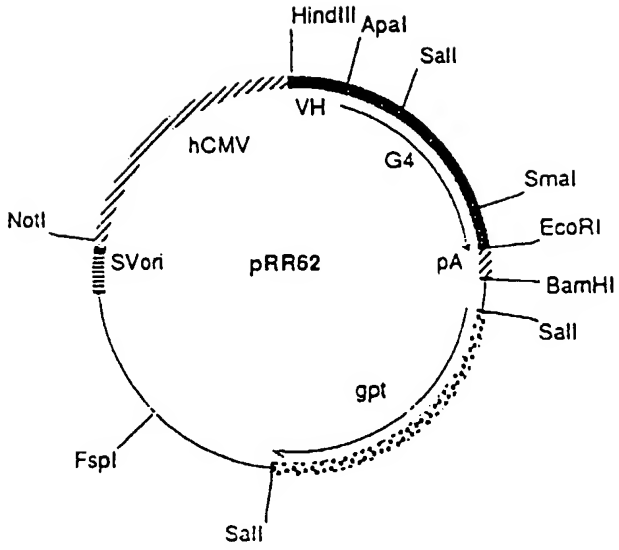

HindIII
ApaI
SalI
VH
hCMV
G4
SmaI
EcoRI
NotI
SVori
pRR62
pA
BamHI
SalI
gpt
FspI
SalI

FIG. 1

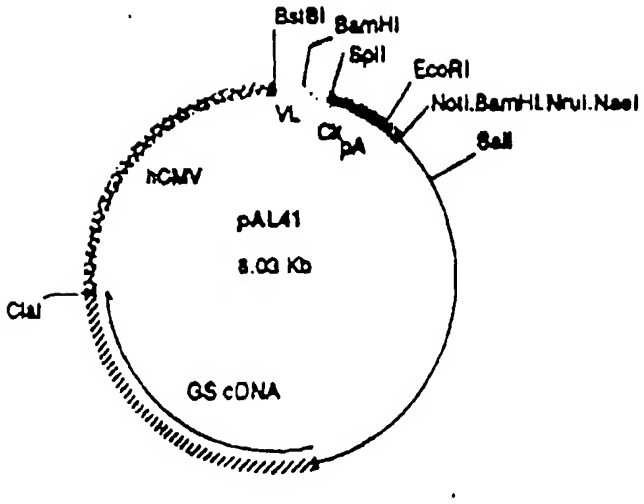

BstBI
BamHI
SpII
EcoRI
NotI.BamHI.NruI.NaeI
SalI
VL
CK
pA
hCMV
pAL41
8.00 Kb
ClaI
GS cDNA

FIG. 2

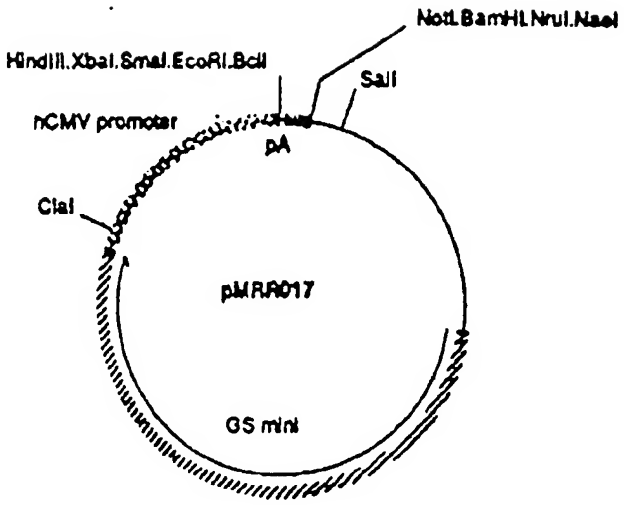

NotI.BamHI.NruI.NaeI
HindIII.XbaI.SmaI.EcoRI.BclI
SalI
hCMV promoter
pA
ClaI
pMRR017
GS mini

FIG. 3

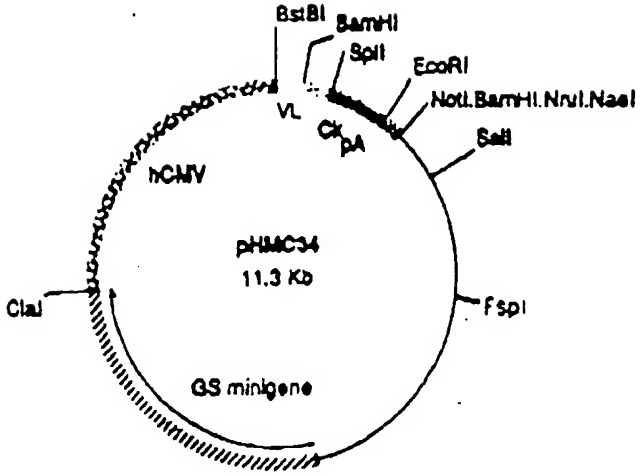

BstBI
BamHI
SpII
EcoRI
NotI.BamHI.NruI.NaeI
SalI
VL
Ck
pA
hCMV
pHMC34
11.3 Kb
ClaI
FspI
GS minigene

FIG. 4

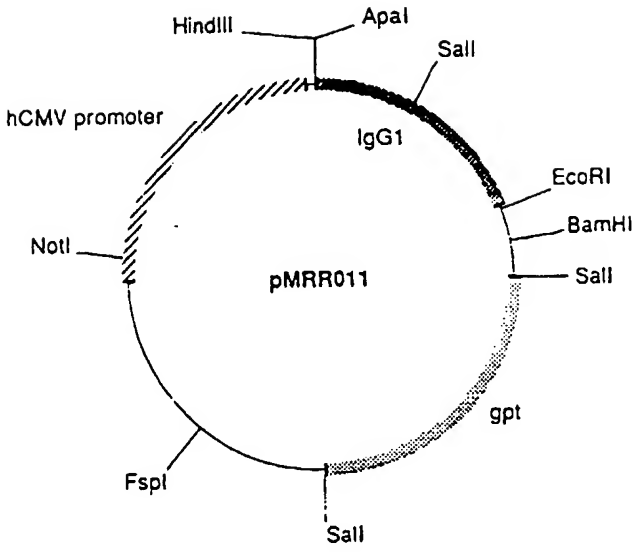

HindIII
ApaI
SalI
hCMV promoter
IgG1
EcoRI
BamHI
NotI
pMRR011
SalI
gpt
FspI
SalI

FIG. 5

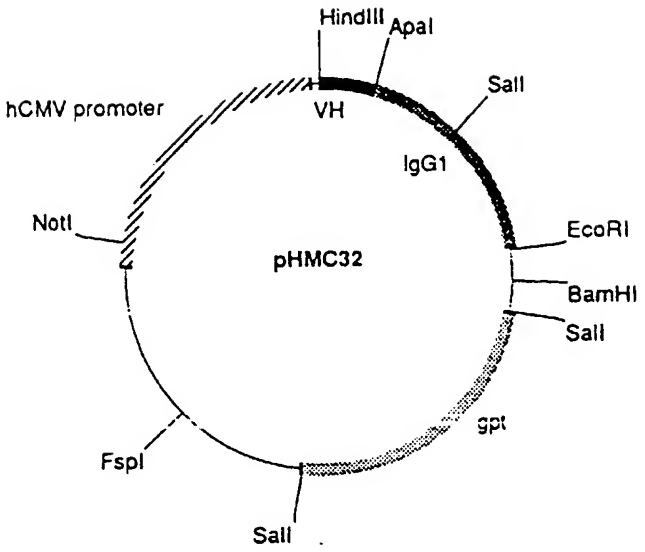

HindIII
ApaI
SalI
hCMV promoter
VH
IgG1
NotI
EcoRI
pHMC32
BamHI
SalI
gpt
FspI
SalI

FIG. 6

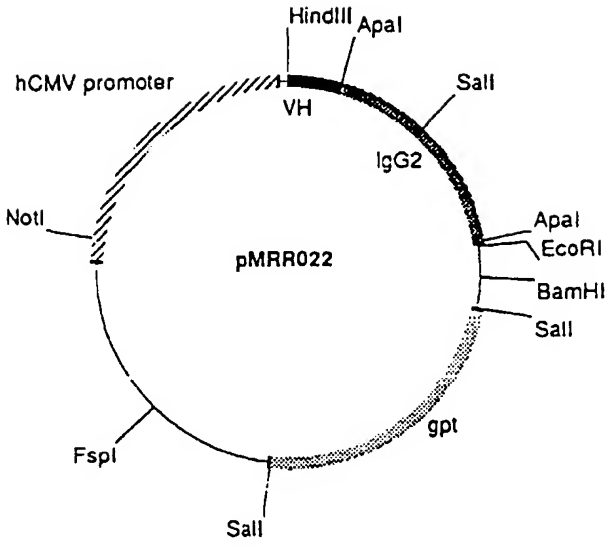

HindIII
ApaI
hCMV promoter
VH
SalI
IgG2
ApaI
EcoRI
NotI
pMRR022
BamHI
SalI
gpt
FspI
SalI

FIG. 7

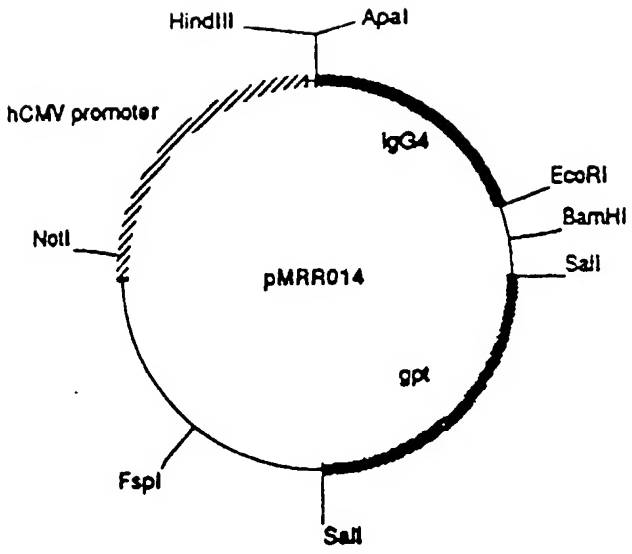

HindIII
ApaI
hCMV promoter
IgG4
EcoRI
BamHI
SalI
NotI
pMRR014
gpt
FspI
SalI

FIG. 8

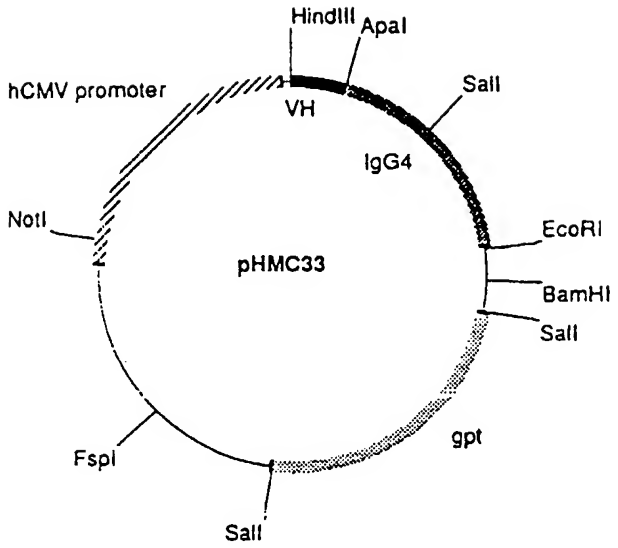

HindIII
ApaI
hCMV promoter
VH
SalI
IgG4
NotI
pHMC33
EcoRI
BamHI
SalI
gpt
FspI
SalI

FIG. 9

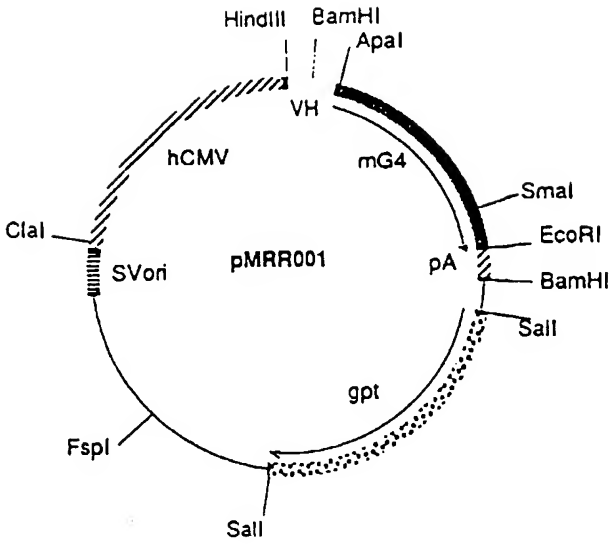

HindIII
BamHI
ApaI
VH
hCMV
mG4
SmaI
EcoRI
BamHI
SalI
ClaI
SVori
pMRR001
pA
gpt
FspI
SalI

FIG. 10

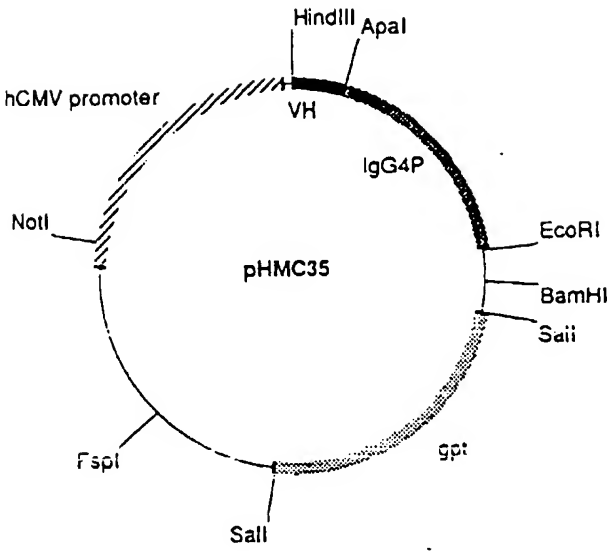

HindIII
ApaI
hCMV promoter
VH
IgG4P
NotI
EcoRI
pHMC35
BamHI
SalI
gpt
FspI
SalI

FIG. 11

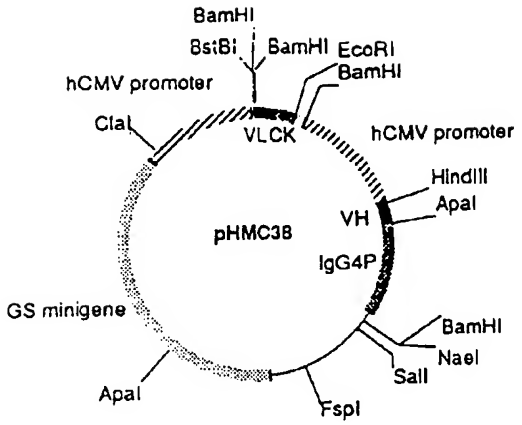


FIG. 12

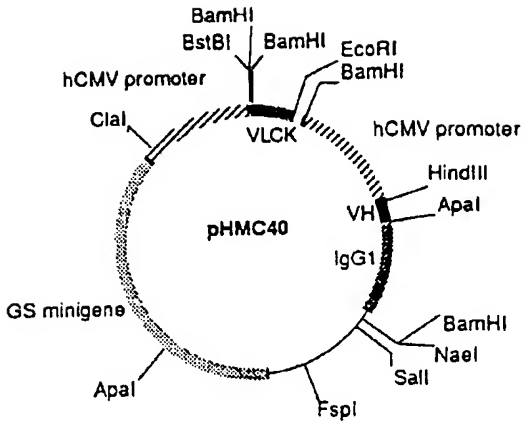


FIG. 13

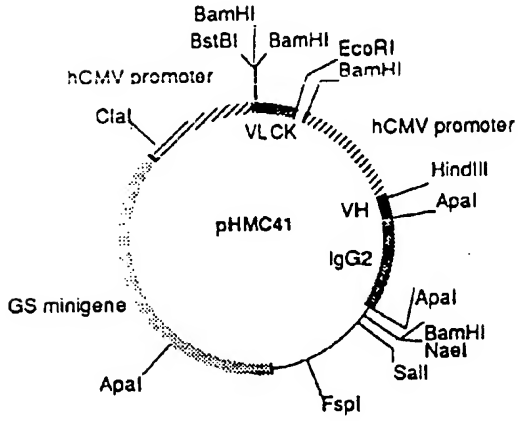


FIG. 14

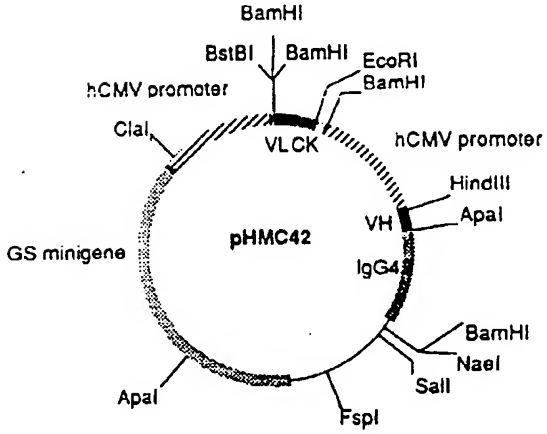


FIG. 15

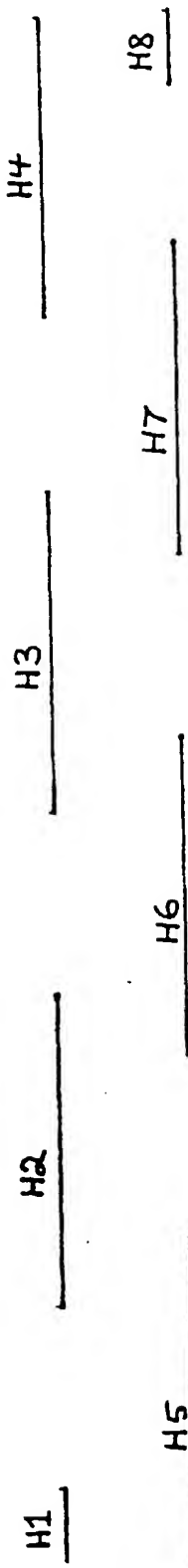


FIG. 16

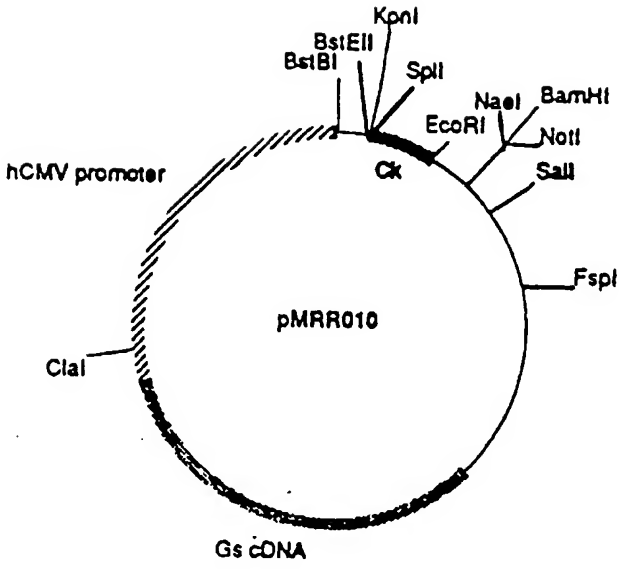

KpnI
BstEII
BstBI
SpII
NaeI
BamHI
EcoRI
NotI
hCMV promoter
Ck
SalI
FspI
pMRR010
ClaI
Gs cDNA

FIG. 19

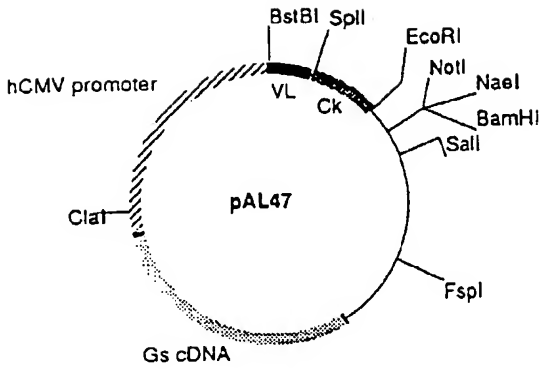


FIG. 20

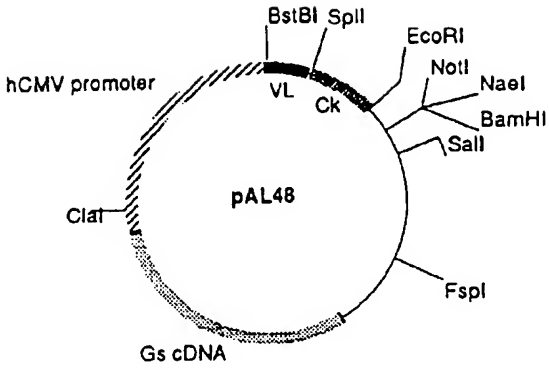


FIG. 21

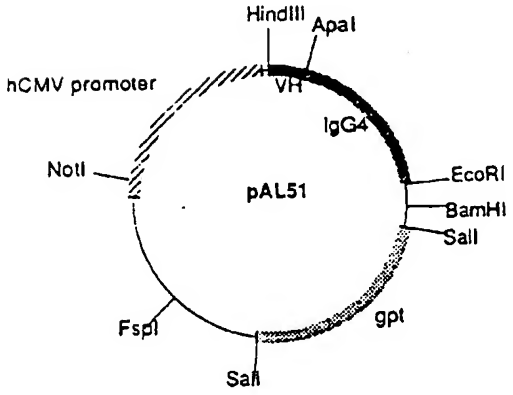


FIG. 17

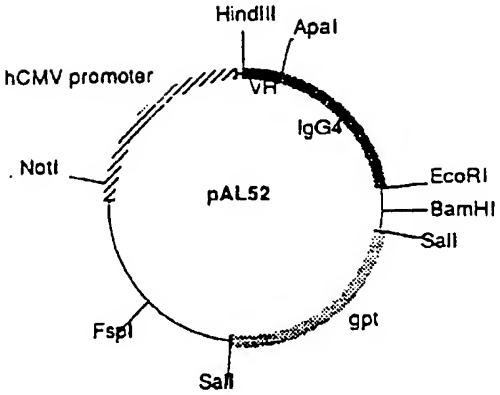


FIG. 18

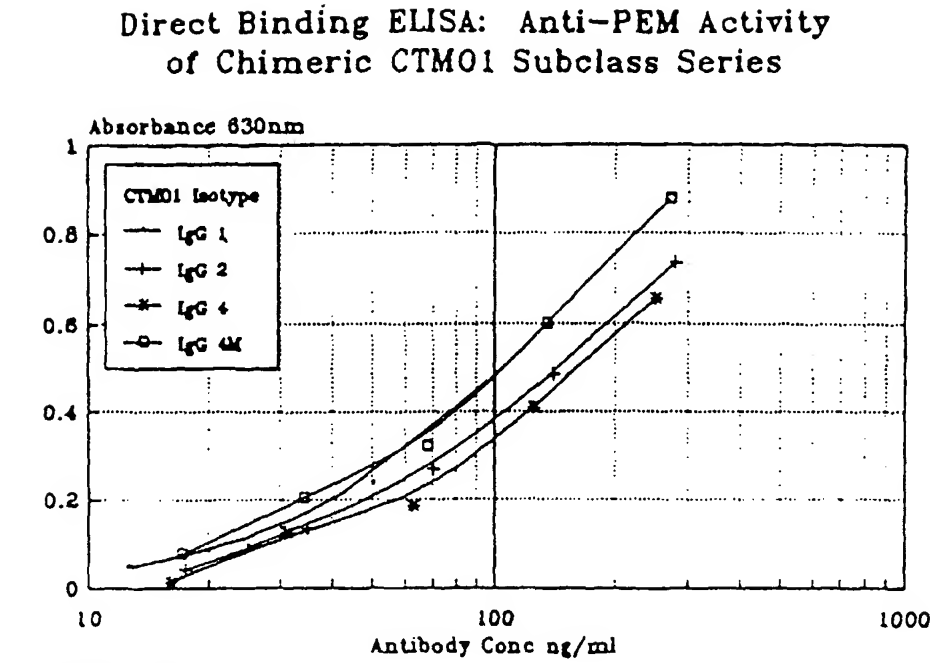

Direct Binding ELISA: Anti-PEM Activity
of Chimeric CTM01 Subclass Series
Absorbance 630nm
1
0.8
0.6
0.4
0.2
0
CTM01 Isotype
IgG 1
IgG 2
IgG 4
IgG 4M
10
100
1000
Antibody Conc ng/ml
A. LAWSON 4.2.91

FIG. 22

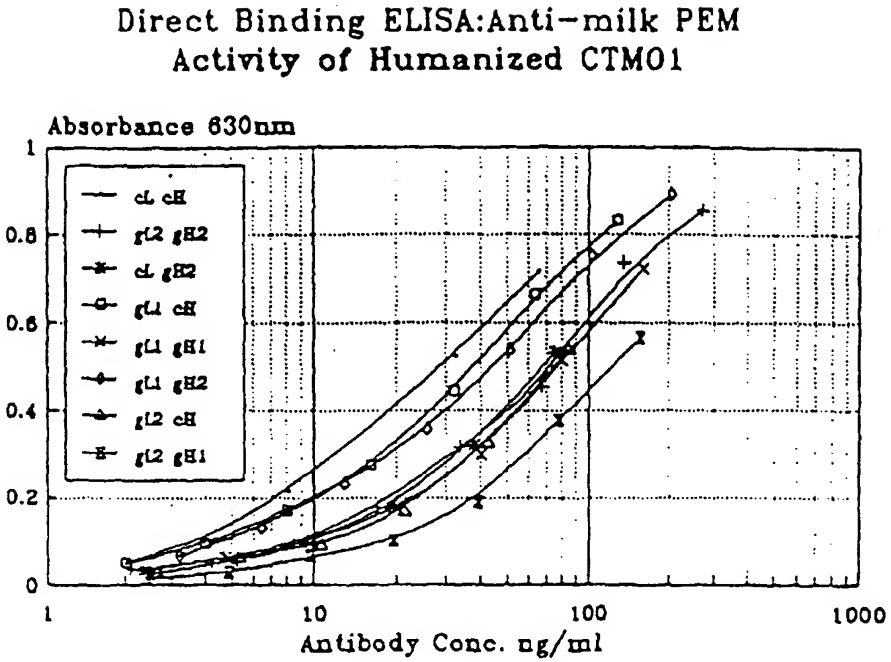

Direct Binding ELISA:Anti-milk PEM
Activity of Humanized CTM01
Absorbance 630nm
1
0.8
0.6
0.4
0.2
0
cL cH
γ2 γH2
cL γH2
γ1 cH
γ1 γH1
γ1 γH2
γ2 cH
γ2 γH1
1
10
100
1000
Antibody Conc. ng/ml

FIG. 23

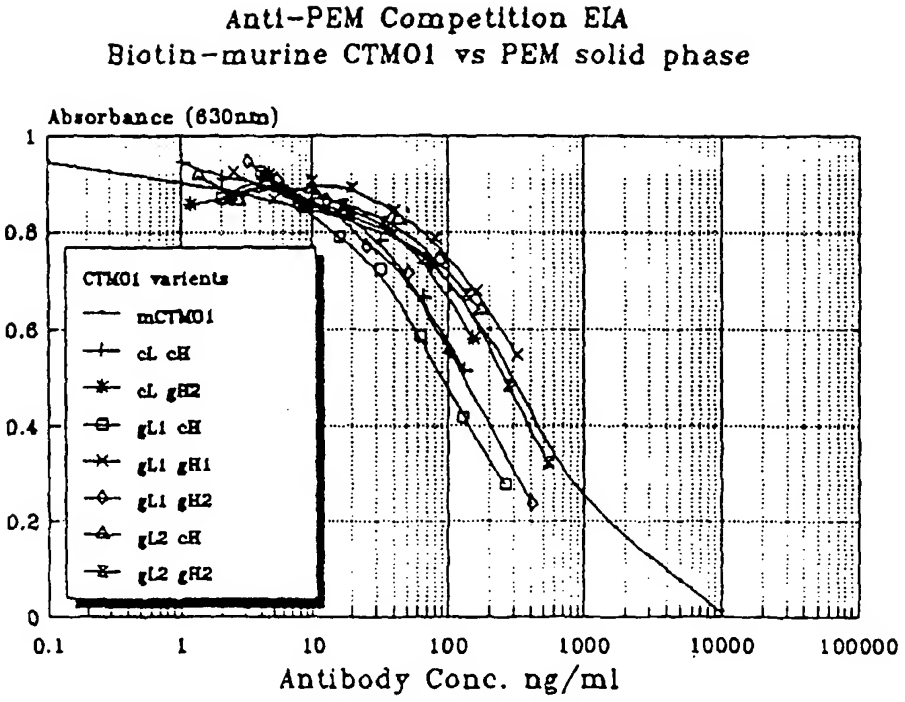

Anti-PEM Competition EIA
Biotin-murine CTM01 vs PEM solid phase
Absorbance (630nm)
1
0.8
0.6
0.4
0.2
0
CTM01 variants
mCTM01
cL cH
cL gH2
gL1 cH
gL1 gH1
gL1 gH2
gL2 cH
gL2 gH2
0.1
1
10
100
1000
10000
100000
Antibody Conc. ng/ml

FIG. 24

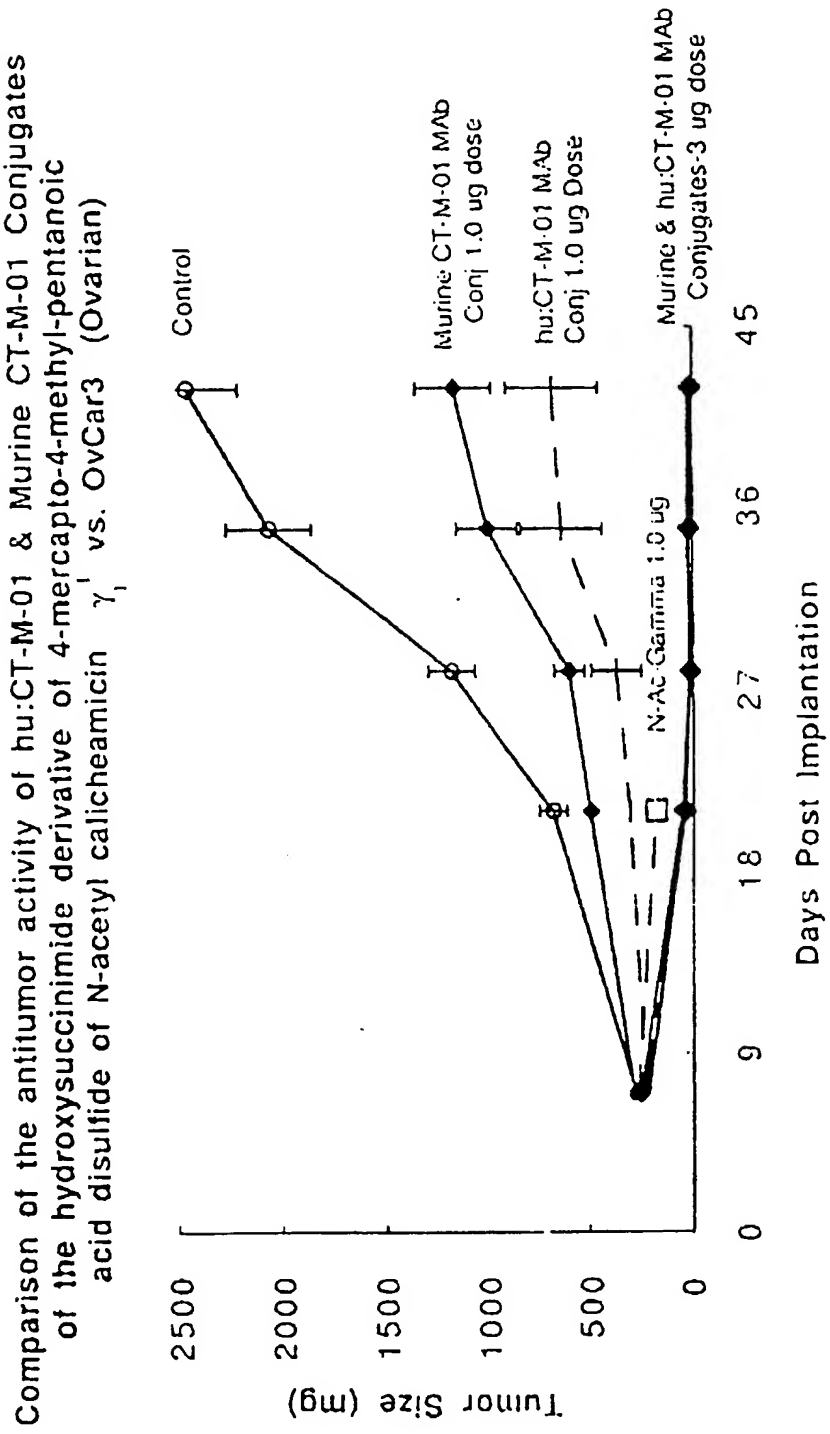

Comparison of the antitumor activity of hu:CT-M-01 & Murine CT-M-01 Conjugates of the hydroxysuccinimide derivative of 4-mercapto-4-methyl-pentanoic acid disulfide of N-acetyl calicheamicin γ_1^I vs. OvCar3 (Ovarian)
Tumor Size (mg)
2500
2000
1500
1000
500
0
0
9
18
27
36
45
Days Post Implantation
Control
Murine CT-M-01 MAb Conj 1.0 ug dose
hu:CT-M-01 MAb Conj 1.0 ug Dose
N-Ac-Gamma 1.0 ug
Murine & hu:CT-M-01 MAb Conjugates-3 ug dose

FIG. 25